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(54) Title: SINGLE NUCLEOTIDE POLYMORPHISMS IN MITOCHONDRIAL GENES THAT SEGREGATE WITH ALZHEIMER'S DISEASE

(57) Abstract

Compositions and methods based on determination of single nucleotide polymorphisms in mtDNA or homoplasmic mtDNA mutations are provided that are useful for detecting the presence of or risk for having Alzheimer's disease (AD), and for identifying agents suitable for treating AD.

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SINGLE NUCLEOTIDE POLYMORPHISMS IN MITOCHONDRIAL GENES THAT SEGREGATE WITH ALZHEIMER'S DISEASE

TECHNICAL FIELD

The present invention relates generally to Alzheimer's disease and, more specifically, to compositions and methods for detecting predisposition to such diseases by detecting single nucleotide polymorphisms in mitochondrial DNA.

BACKGROUND OF THE INVENTION

A number of degenerative diseases are thought to be caused by, or are associated with, alterations in mitochondrial function. These diseases include Alzheimer's Disease, diabetes mellitus, Parkinson's Disease, Huntington's disease, dystonia, Leber's hereditary optic neuropathy, schizophrenia, and myodegenerative disorders such as "mitochondrial encephalopathy, lactic acidosis, and stroke" (MELAS), and "myoclonic epilepsy ragged red fiber syndrome" (MERRF). Other diseases involving altered metabolism or respiration within cells may also be regarded as diseases associated with altered mitochondrial function.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is characterized by loss and/or atrophy of neurons in discrete regions of the brain, and that is accompanied by extracellular deposits of β-amyloid and the intracellular accumulation of neurofibrillary tangles. It is a uniquely human disease, affecting over 13 million people worldwide. It is also a uniquely tragic disease. Many individuals who have lived normal, productive lives are slowly stricken with AD as they grow older, and the disease gradually robs them of their memory and other mental faculties. Eventually, they cease to recognize family and loved ones, and they often require continuous care until their eventual death.

There is evidence to suggest that the genetic basis of at least some diseases associated with altered mitochondrial function resides in mitochondrial DNA rather than in extramitochondrial DNA such as that found in the nucleus. For example, noninsulin dependent diabetes mellitus (NIDDM) exhibits a predominantly maternal pattern of inheritance and is also present in diseases known to be based on a mitochondrial DNA (mtDNA) defect. Approximately 1.5% of all diabetic individuals, for instance, harbor a mutation at mtDNA position 3243 in the mitochondrial gene encoding leucyl-tRNA ($tRNA^{Leu}$). This mutation is known as the MELAS (mitochondrial encephalopathy, lactic acidosis and stroke) mutation. (Gerbitz et al., *Biochim. Biophys. Acta 1271*:253-260, 1995.) Similar theories have been advanced for

analogous relationships between mtDNA mutations and other diseases associated with altered mitochondrial function, including but not limited to Alzheimer's Disease (AD), Huntington's Disease (HD), Parkinson's Disease (PD), dystonia, Leber's hereditary optic neuropathy (LHON), schizophrenia, and myoclonic epilepsy ragged red fiber syndrome (MERRF). Identification of such mutations and their functional consequences may provide targets for development of diagnostic and/or therapeutic agents.

Mitochondria are the subcellular organelles that manufacture bioenergetically essential adenosine triphosphate (ATP) by oxidative phosphorylation. Functional mitochondria contain gene products encoded by mitochondrial genes situated in mitochondrial DNA (mtDNA) and by extramitochondrial genes not situated in the circular mitochondrial genome. The 16.5 kb mtDNA encodes 22 tRNAs, two ribosomal RNAs (12s and 16s rRNA) and only 13 enzymes of the electron transport chain (ETC), the elaborate multi-complex mitochondrial assembly where, for example, respiratory oxidative phosphorylation takes place. (See, *e.g.*, Wallace et al., in *Mitochondria & Free Radicals in Neurodegenerative Diseases*, M.F. Beal, N. Howell and I. Bodis-Wollner, eds., 1997 Wiley-Liss, Inc., New York, pp. 283-307, and references cited therein; see also, *e.g.*, Scheffler, I.E., *Mitochondria*, 1999 Wiley-Liss, Inc., New York.) Mitochondrial DNA includes gene sequences encoding a number of ETC components, including seven subunits of NADH dehydrogenase, also known as ETC Complex I (ND1, ND2, ND3, ND4, ND4L, ND5 and ND6); one subunit of Complex III (ubiquinol: cytochrome c oxidoreductase, Cytb); three cytochrome c oxidase (Complex IV) subunits (COX1, COX2 and COX3); and two proton-translocating ATP synthase (Complex V) subunits (ATPase6 and ATPase8). The overwhelming majority of mitochondrial structural and functional proteins are encoded by extramitochondrial, and in most cases presumably nuclear, genes. Accordingly, mitochondrial and extramitochondrial genes may interact directly, or indirectly via gene products and their downstream intermediates, including metabolites, catabolites, substrates, precursors, cofactors and the like. Alterations in mitochondrial function, for example impaired electron transport activity, defective oxidative phosphorylation or increased free radical production, may therefore arise as the result of defective mtDNA, defective extramitochondrial DNA, defective mitochondrial or extramitochondrial gene products, defective downstream intermediates or a combination of these and other factors.

In the case of AD, efforts to demonstrate relationships between mtDNA mutations and disease typically involve preparation of mtDNA followed by restriction

fragment length polymorphism (RFLP) or related analysis (see, *e.g.*, Shoffner et al. 1993 *Genomics* 17:171; Petruzzella et al., 1992 *Biochem. Biophys. Res. Commun.* 186:491; Kosel et al., 1994 *Biochem. Biophys. Res. Commun.* 203:745; Hutchin et al., 1995 *Proc. Nat. Acad. Sci. USA* 18:6892; Brown et al., 1996 *Am J. Med. Genet.* 61:283; Wragg et al., 1995 *Neurosci. Lett.* 201:107; Zsurka et al., 1998 *Biol. Psychiatry* 44:371; Hutchin et al., 1997 *Biochem. Biophys. Res. Commun.* 241:221; Hamblet et al., 1997 *Mutat. Res.* 379:253; Egensperger et al., 1997 *Neuropathol. Appl Neurobiol.* 23:315; Lin et al., 1992 Biochem. Biophys. Res. Commun. 182:238; Tanno et al., 1998 *Neurobiol. Of Aging,* 19(1S):S47; WO 94/09162) relative to RFLP patterns predicted by the sequence of wildtype human mtDNA (*e.g.*, Anderson et al., 1981 *Nature* 290:457). Such approaches, however, rely upon the occurrence of a mutation such as a nucleotide substitution at particular positions within the mtDNA sequence, such that the restriction fragment pattern profile generated by selected restriction endonucleases is altered.

Clearly there is a need for improved compositions and methods for the detection of AD, and for identifying therapeutic agents that will be useful in the treatment of AD. Regardless of whether a defect underlying AD may have mitochondrial or extramitochondrial origins, and regardless of whether a defect underlying altered mitochondrial function has been identified, the present invention provides methods that are useful for determining the risk or presence of AD, and for identifying agents that are suitable for treating this disease. In particular, as is elaborated herein below, the present invention provides compositions and methods for the detection of AD by identification of unusual single nucleotide polymorphisms or homoplasmic mtDNA mutations, and other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention is directed to compositions and methods useful for detecting AD and involving identification of single nucleotide polymorphisms or homoplasmic mutations in mitochondrial DNA (mtDNA). It is therefore an aspect of the invention to provide a method for determining the risk for or presence of Alzheimer's disease in a first subject suspected of having or being at risk for having such a disease, comprising determining the presence or absence of at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease in each of a first and a second biological sample comprising mitochondrial DNA, the first biological sample being obtained from the first subject and the second sample being obtained from a second subject known to be free of a risk or presence of a

disease associated with altered mitochondrial function, wherein the presence of at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease in the first biological sample and the absence of a mitochondrial single nucleotide polymorphism at a corresponding nucleotide in the second biological sample indicates an increased risk of Alzheimer's disease, and therefrom determining the risk or presence of Alzheimer's disease.

In a related embodiment, the mitochondrial DNA in the first sample is amplified and the mitochondrial DNA in the second sample is amplified. In another embodiment, the step of determining comprises contacting each of the first and second biological samples with an oligonucleotide primer having a nucleotide sequence that is complementary to a sequence present in the mitochondrial DNA of the first sample and present in the mitochondrial DNA of the second sample, under conditions and for a time sufficient to allow hybridization of the primer to the mitochondrial DNA; and detecting hybridization and extension of the primer to the mitochondrial DNA of the first sample to produce a first product and hybridization and extension of the primer to the mitochondrial DNA of the second sample to produce a second product distinguishable from the first product, and therefrom determining the presence or absence of at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease. In certain embodiments, the mitochondrial DNA in the first sample is amplified and the mitochondrial DNA in the second sample is amplified.

In another embodiment, at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease and present in the first biological sample and that is absent at a corresponding nucleotide in the second biological sample is present in a mitochondrial DNA region that is a D-loop, a mitochondrial rRNA gene, a mitochondrial NADH dehydrogenase gene, a mitochondrial tRNA gene, a mitochondrial cytochrome c oxidase gene, a mitochondrial ATP synthase gene or a mitochondrial cytochrome b gene. In another embodiment, at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease and present in the first biological sample and that is absent at a corresponding nucleotide in the second biological sample is present in a mitochondrial DNA region that is a D-loop, a mitochondrial rRNA gene, a mitochondrial NADH dehydrogenase gene, a mitochondrial tRNA gene, a mitochondrial cytochrome c oxidase gene, a mitochondrial ATP synthase gene or a mitochondrial cytochrome b gene. In another embodiment, at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease and present in the first biological sample and that is absent at a corresponding nucleotide in the second

biological sample is present in a mitochondrial DNA region that is a mitochondrial NADH dehydrogenase gene, a mitochondrial cytochrome c oxidase gene, a mitochondrial ATP synthase gene or a mitochondrial cytochrome b gene, and the single nucleotide polymorphism is a non-synonymous nucleotide substitution. In another embodiment, at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease and is present in the first biological sample and that is absent at a corresponding nucleotide in the second biological sample is present in a mitochondrial DNA region that is a mitochondrial NADH dehydrogenase gene, a mitochondrial cytochrome c oxidase gene, a mitochondrial ATP synthase gene or a mitochondrial cytochrome b gene, and the single nucleotide polymorphism is a synonymous nucleotide substitution.

In certain embodiments of the present invention, at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease and is present in the first biological sample and that is absent at a corresponding nucleotide in the second biological sample is a mitochondrial single nucleotide polymorphism located at a nucleotide that corresponds to a nucleotide position of SEQ ID NO:1 that is position 72, 114, 146, 185, 189, 199, 204, 207, 228, 236, 239, 456, 462, 482, 489, 497, 500, 516, 522, 523, 547, 593, 669, 960, 1007, 1243, 1393, 1719, 1809, 2352, 2483, 2702, 2851, 3197, 3333, 3336, 3348, 3394, 3398, 3423, 3505, 3559, 3915, 3992, 4024, 4095, 4216, 4336, 4529, 4727, 4793, 4917, 4991, 5004, 5046, 5228, 5315, 5418, 5426, 5460, 5461, 5516, 5554, 5634, 5656, 5773, 6182, 6221, 6341, 6367, 6371, 6489, 7184, 7325, 7621, 7768, 7787, 7789, 7864, 7895, 7963, 8149, 8251, 8269, 8276-8284, 8470, 8485, 8508, 8602, 8697, 8752, 8901, 8994, 9123, 9254, 9362, 9380, 9477, 9554, 9708, 9804, 9861, 10034, 10044, 10238, 10463, 10589, 10978, 11065, 11251, 11253, 11272, 11470, 11527, 11611, 11674, 11812, 11914, 11947, 12414, 12501, 12609, 12705, 12954, 13111, 13194, 13212, 13368, 13617, 13780, 13966, 14020, 14148, 14178, 14179, 14182, 14212, 14233, 14470, 14582, 14905, 15028, 15043, 15191, 15299, 15380, 15553, 15607, 15758, 15790, 15808, 15833, 15884, 15924, 15928, 16069, 16086, 16093, 16126, 16129, 16145, 16147, 16172, 16174, 16182, 16183, 16189, 16192, 16193, 16223, 16224, 16234, 16235, 16239, 16248, 16256, 16261, 16270, 16278, 16290, 16292, 16293, 16294, 16298, 16300, 16304, 16309, 16311, 16320, 16355, 16362, 16391, 16482 or 16524.

In another embodiment, at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease and is present in the first biological sample and that is absent at a corresponding nucleotide in the second biological sample is a mitochondrial single nucleotide polymorphism located at a

nucleotide that corresponds to a nucleotide position of SEQ ID NO:1 that is position 709, 930, 960, 980, 1189, 1243, 1700, 1719, 1809, 1811, 1888, 2098, 2158, 2259, 2352, 3010, 3197, 669, 789, 793, 870, 980, 1007, 1243, 1393, 1709, 1719, 2156, 2294, 2483, 2581, 2851, 6366 or 12954.

In certain other embodiments the invention provides a method for determining the risk for or presence of Alzheimer's disease in a subject, comprising: determining the presence of at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease in a biological sample comprising mitochondrial DNA from the subject. In certain embodiments at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease is present in a mitochondrial DNA region that is a D-loop, a mitochondrial rRNA gene, a mitochondrial NADH dehydrogenase gene, a mitochondrial tRNA gene, a mitochondrial cytochrome c oxidase gene, a mitochondrial ATP synthase gene or a mitochondrial cytochrome b gene. In certain other embodiments at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease is present in a mitochondrial DNA region that is a mitochondrial NADH dehydrogenase gene, a mitochondrial cytochrome c oxidase gene, a mitochondrial ATP synthase gene or a mitochondrial cytochrome b gene, and the single nucleotide polymorphism is a non-synonymous nucleotide substitution. In certain other embodiments at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease is present in a mitochondrial DNA region that is a mitochondrial NADH dehydrogenase gene, a mitochondrial cytochrome c oxidase gene, a mitochondrial ATP synthase gene or a mitochondrial cytochrome b gene, and the single nucleotide polymorphism is a synonymous nucleotide substitution.

In another embodiment, at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease is a mitochondrial single nucleotide polymorphism located at a nucleotide that corresponds to a nucleotide position of SEQ ID NO:1 that is position 72, 114, 146, 185, 189, 199, 204, 207, 228, 236, 239, 456, 462, 482. 489, 497, 500, 516, 522, 523, 547, 593, 669, 960, 1007, 1243, 1393, 1719, 1809, 2352, 2483, 2702, 2851, 3197, 3333, 3336, 3348, 3394, 3398, 3423, 3505, 3559, 3915, 3992, 4024, 4095, 4216, 4336, 4529, 4727, 4793, 4917, 4991, 5004, 5046, 5228, 5315, 5418, 5426, 5460, 5461, 5516, 5554, 5634, 5656, 5773, 6182, 6221, 6341, 6367, 6371, 6489, 7184, 7325, 7621, 7768, 7787, 7789, 7864, 7895, 7963, 8149, 8251, 8269, 8276-8284, 8470, 8485, 8508. 8602, 8697, 8752, 8901, 8994, 9123, 9254, 9362, 9380, 9477, 9554, 9708, 9804, 9861, 10034, 10044, 10238, 10463, 10589, 10978, 11065, 11251, 11253, 11272, 11470, 11527, 11611, 11674, 11812, 11914, 11947,

12414, 12501, 12609, 12705, 12954, 13111, 13194, 13212, 13368, 13617, 13780, 13966, 14020, 14148, 14178, 14179, 14182, 14212, 14233, 14470, 14582, 14905, 15028, 15043, 15191, 15299, 15380, 15553, 15607, 15758, 15790, 15808, 15833, 15884, 15924, 15928, 16069, 16086, 16093, 16126, 16129, 16145, 16147, 16172, 16174, 16182, 16183, 16189, 16192, 16193, 16223, 16224, 16234, 16235, 16239, 16248, 16256, 16261, 16270, 16278, 16290, 16292, 16293, 16294, 16298, 16300, 16304, 16309, 16311, 16320, 16355, 16362, 16391, 16482 or 16524.

In certain embodiments, at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease is a mitochondrial single nucleotide polymorphism located at a nucleotide that corresponds to a nucleotide position of SEQ ID NO:1 that is position 709, 930, 960, 980, 1189, 1243, 1700, 1719, 1809, 1811, 1888, 2098, 2158, 2259, 2352, 3010, 3197, 669, 789, 793, 870, 980, 1007, 1243, 1393, 1709, 1719, 2156, 2294, 2483, 2581, 2851, 6366 or 12954.

In another aspect, the invention provides a method of determining if an agent is likely to cause, contribute to the pathology of, or exacerbate Alzheimer's disease, comprising contacting a cell with a candidate agent, conducting an assay of a mitochondrial nucleic acid to determine if the mitochondrial nucleic acid contains one or more single nucleotide polymorphisms after being contacted with the agent, wherein the nucleic acid is present in or derived from the cell and the one or more single nucleotide polymorphisms segregate with Alzheimer's disease.

It is another aspect of the invention to provide a method of determining if an agent is likely to cause, contribute to the pathology of, or exacerbate Alzheimer's disease, comprising the steps of contacting a first cell with a candidate agent; incubating a second cell, that has not been contacted with the agent, and the first cell, under equivalent conditions; conducting an assay of one or more mitochondrial nucleic acids to determine if the mitochondrial nucleic acids contain one or more single nucleotide polymorphisms, wherein the nucleic acids are present in or derived from the cells and the single nucleotide polymorphisms segregate with Alzheimer's disease, wherein the presence of one or more of the mutations in the mitochondrial nucleic acids present in or derived from the first cell, and the absence of one or more of the mutations in the mitochondrial nucleic acids present in or derived from the second cell, indicates that the agent is likely to cause, contribute to the pathology of, or exacerbate Alzheimer's disease.

In another aspect the present invention provides a nucleic acid array comprising a plurality of isolated nucleic acid molecules immobilized on a solid support, wherein the isolated nucleic acid molecules comprise all or a portion of the

nucleic acid sequence set forth in SEQ ID NO:1 in which at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease is present. In certain embodiments, the mitochondrial single nucleotide polymorphism is located at a nucleotide that corresponds to a nucleotide position of SEQ ID NO:1 that is any of the above recited position numbers. In other embodiments, the step of determining comprises contacting each of the first and second biological samples with an oligonucleotide primer comprising all or a portion of the nucleic acid sequence set forth in SEQ ID NO:1 in which at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease is present, under conditions and for a time sufficient to allow hybridization of the primer to the mitochondrial DNA; and comparing an amount of hybridization of the oligonucleotide primer to the mitochondrial DNA of the first sample to an amount of hybridization of the primer to the mitochondrial DNA of the second sample, and therefrom determining the presence or absence of at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease. In certain embodiments, the mitochondrial single nucleotide polymorphism is located at a nucleotide that corresponds to a nucleotide position of SEQ ID NO:1 that is any of the above recited position numbers. In certain other embodiments, the step of determining comprises contacting each of the first and second biological samples with a nucleic acid array comprising a plurality of isolated nucleic acid molecules immobilized on a solid support, wherein the isolated nucleic acid molecules comprise all or a portion of the nucleic acid sequence set forth in SEQ ID NO:1 in which at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease is present, under conditions and for a time sufficient to allow hybridization of mitochondrial DNA to the isolated nucleic acid molecules; and comparing an amount of hybridization of the mitochondrial DNA of the first sample to the nucleic acid array to an amount of hybridization of the mitochondrial DNA of the second sample to the nucleic acid array, and therefrom determining the presence or absence of at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease. In certain embodiments, the mitochondrial single nucleotide polymorphism is located at a nucleotide that corresponds to a nucleotide position of SEQ ID NO:1 that is any of the above recited position numbers.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings. In addition, various references are set forth herein which describe in more detail certain aspects of this invention, and are therefore incorporated by reference in their entireties.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts mitochondrial rRNA gene mutations associated with AD.

Figure 2 shows oligonucleotide primer extension reaction results from AD and control cybrid cells.

Figure 3 depicts mitochondrial ETC Complex IV activity in AD and control cybrid cells.

Figure 4 shows western immunoblot analysis of COX subunits in AD and control cybrid cells, and in SH-SY5Y neuroblastoma cells.

Figure 5 illustrates the effect on ROS production of agents that interfere with radical scavenging enzymes in AD and control cybrid cells.

Figure 6 shows the oxidative buffering capacity of radical scavenging enzymes in AD-1 cybrid cells relative to mixed control cybrid cells, and also shows the relative levels of gene expression for two of the enzymes, Cu/Zn SOD and Mn SOD.

Figure 7 shows differences between AD and control subjects in mtDNA single nucleotide polymorphisms in mitochondrial rRNA genes as a function of age.

Figure 8 depicts AD-associated and control SNPs according to haplogroup.

Figure 9 depicts AD-associated and control non-synonymous SNPs according to mtDNA gene loci. ND, NADH dehydrogenase; CO, cytochrome c oxidase; AT, ATP synthase; CYB, cytochrome b.

Figure 10 depicts AD-associated and control non-synonymous SNPs according to mtDNA gene loci. Abbreviations as in Fig. 9.

Figure 11 depicts AD-associated and control synonymous SNPs according to mtDNA gene loci. Abbreviations as in Fig. 9.

Figure 12 depicts AD-associated and control SNPs according to each of the 22 mitochondrial tRNA genes of mtDNA indicated by amino acid or mitochondrial genetic code specificity (F, V, LUUR, I, Q, M, W, A, N, C, Y, SUCN, D, K, G, R, H, SAGY, LCUR, E, T, P; for additional information on the mitochondrial genetic code, see, *e.g.*, Steele et al., 1996 *Proc. Nat. Acad. Sci.* USA 93:5253 and references cited therein.)

Figure 13 summarizes AD-associated and control SNPs according to mtDNA regions.

Figure 14 summarizes the frequencies per subject of AD-associated and control SNPs according to mtDNA regions.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed generally to compositions and methods for diagnosing the risk or presence of Alzheimer's disease (AD), and to compositions and methods for the identification of agents that may be suitable for treating AD.

According to the present invention, alterations in mitochondrial DNA (mtDNA) as described herein provide a novel and useful parameter for diagnosing the risk or presence of AD, and for identifying agents that may be suitable for treating this disease. Such alterations may include, for instance, single nucleotide polymorphisms (SNPs) or homoplasmic mtDNA mutations (*see, e.g.*, Scheffler, I.E., *Mitochondria*, 1999, Wiley-Liss, Inc., New York, pp. 286-287). The present invention is therefore directed in pertinent part to mutations that are associated with AD, including but not limited to SNPs or homoplasmic mtDNA mutations that occur at specific positions in mtDNA in certain embodiments. SNPs or homoplasmic mtDNA mutations that in certain other embodiments occur with altered frequencies (*e.g.*, frequencies that are increased or decreased in a statistically significant manner) in subjects having or being at risk for having AD relative to subjects known to be free of a risk for having AD, or SNPs or homoplasmic mtDNA mutations that in certain other embodiments occur with altered frequencies (*e.g.*, frequencies that are increased or decreased in a statistically significant manner) in patient populations having or being at risk for having AD relative to populations known to be free of a risk for having AD.

A biological sample for use according to the present invention, containing mtDNA as provided herein, may comprise any tissue or cell preparation in which mitochondrially derived nucleic acids (*e.g.*, mtDNA) are present. Compositions and methods useful for obtaining and detecting mtDNA are provided, for example, in U.S. Patent Nos. 5,565,323 and 5,840,493. Biological samples may be provided by obtaining a blood sample, biopsy specimen, tissue explant, organ culture or any other tissue or cell preparation from a subject or a biological source. The subject or biological source may be a human or non-human animal, a primary cell culture or culture adapted cell line, including but not limited to genetically engineered cell lines that may contain chromosomally integrated or episomal recombinant nucleic acid sequences, immortalized or immortalizable cell lines, somatic cell hybrid or cytoplasmic hybrid "cybrid" cell lines (see, *e.g.*, U.S. Patent No. 5,888,498), differentiated or differentiatable cell lines, transformed cell lines and the like. In certain embodiments of the invention, the subject or biological source may be suspected of having or being at risk for having a disease associated with altered mitochondrial function (*e.g.*, AD), and in certain embodiments of the invention, the subject or

biological source may be known to be free of a risk or presence of such a disease. For example, and according to non-limiting theory, in certain embodiments it may be desirable to use as a subject or biological source a control individual, typically an age- and/or sex-matched individual, a healthy individual or an individual appropriate as a control for a subject suspected of having or being at risk for AD by any number of other biological, physiological, immunological, pharmacological, pathological, neurological or other biomedical criteria, or the like. Those having ordinary skill in the art are familiar with design and selection of such parameters for clinical correlation. For instance, in certain embodiments it may be desirable to identify such a control individual who is believed to be free of any AD-associated signs and symptoms as described below, and in certain other embodiments, a control individual may share a mitochondrial genetic relationship to a subject suspected of being at risk for AD, such as the mother or sibling of the subject (*see, e.g.*, Scheffler, 1999, *supra*). In certain other embodiments of the present invention the subject or biological source is at least 64 years of age, and in certain other embodiments the subject or biological source is at least 75 years of age. In certain preferred embodiments the subject or biological source is at least 85 years of age.

In certain other preferred embodiments it may be desirable to determine whether a subject or biological source falls within clinical parameters indicative of Alzheimer's disease (AD). Signs and symptoms of AD accepted by those skilled in the art may be used to so designate a subject or biological source, for example clinical signs referred to in McKhann et al. (*Neurology 34*:939, 1984, National Institute of Neurology, Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association Criteria of Probable AD, NINCDS-ADRDA) and references cited therein, or other means known in the art for diagnosing AD. Any mtDNA sequence or portion of a mutated mtDNA sequence that corresponds to the human mtDNA sequence disclosed by Anderson et al. (SEQ ID NO:1, 1981 *Nature* 290:457; see also Marzuki et al., 1991 *Human Genet.* 88:139) and revised according to Andrews et al. (1999 *Nature Genetics* 23:147), or a portion thereof or several portions thereof, may be useful in these embodiments of the invention. Examples of human mtDNA point mutations derived from specific mtDNA sequence regions that are useful in these and other embodiments of the invention are disclosed, according to the nucleotide positions at which wildtype and mutant mtDNA differ, in Tables 1-4 and Table 9.

Portions of the mtDNA sequence of SEQ ID NO:1, and portions of a sample mtDNA sequence derived from a biological source or subject as provided herein, are regarded as "corresponding" nucleic acid sequences, regions, fragments or

the like, based on the convention for numbering mtDNA nucleic acid positions according to SEQ ID NO:1 (Anderson et al., *Nature 290*:457, 1981), wherein a sample mtDNA sequence is aligned with the mtDNA sequence of SEQ ID NO:1 such that at least 70%, preferably at least 80% and more preferably at least 90% of the nucleotides in a given sequence of at least 20 consecutive nucleotides of a sequence are identical. For example, a portion of the mtDNA sequence in a biological sample containing mtDNA from a subject suspected of having or being at risk for having AD, or, as another example, a portion of the mtDNA sequence in mtDNA containing at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease as provided herein (*e.g.*, mutated mtDNA), may be aligned with a corresponding portion of the mtDNA sequence of SEQ ID NO:1 using any of a number of alignment procedures and/or tools with which those having ordinary skill in the art will be familiar (*e.g.*, CLUSTAL W, Thompson et al., 1994 *Nucl. Ac. Res.* 22:4673: CAP. www.no.embnet.org/clustalw.html: FASTA/FASTP, Pearson, 1990 *Proc. Nat. Acad. Sci. USA* 85:2444, available from D. Hudson, Univ. of Virginia, Charlottesville, VA). In certain preferred embodiments, a sample mtDNA sequence is greater than 95% identical to a corresponding mtDNA sequence of SEQ ID NO:1. In certain other preferred embodiments, a sample mtDNA sequence is identical to a corresponding mtDNA sequence of SEQ ID NO:1. Those oligonucleotide probes having sequences that are identical in corresponding regions of the mtDNA sequence of SEQ ID NO:1 and sample mtDNA may be identified and selected following hybridization target DNA sequence analysis, to verify the absence of mutations.

According to the present invention and as known in the art, the term "haplotype" refers to a particular combination of genetic markers in a defined region of the mitochondrial chromosome. Such genetic markers include, for example, RFLPs and SNPs. RFLPs (restriction fragment polymorphisms) result from an alteration in a recognition site, often a palindrome, that is specifically cleaved in a site-specific manner by a DNAse known as a restriction enzyme. A SNP (single nucleotide polymorphism) is a change (*e.g.*, a deletion, insertion or substitution) in any single nucleotide base in a region of a genome of interest. In particularly preferred embodiments provided by the instant disclosure, the genome of interest is the mitochondrial genome. Because SNPs vary from individual to individual, they are useful markers for studying the association of a genome. Moreover, because they occur more frequently than other markers such as RFLPs, analysis of SNPs should produce a "higher resolution" picture of disease-associated genetic marker segregation (Weiss, *Genome Res. 8*:691-697, 1998; Gelbert and Gregg, *Curr. Opin. Biotechnol. 8*:669-674, 1997).

The term "haplogroup" refers to a group of haplotypes found in association with one another. Several mitochondrial DNA haplotypes and haplogroups are known in the art, including ten European mtDNA haplogroups as well as discrete Asian, Native American and African mtDNA haplogroups, each identified on the basis of the presence or absence of one or more specific restriction endonuclease recognition sites (see, *e.g.*, Wallace et al., 1999 *Gene* 238:211; Torroni et al., 1996 *Genetics* 144:1835).

Nucleic acid sequences within the scope of the invention include isolated DNA and RNA sequences that specifically hybridize under conditions of moderate or high stringency to mtDNA nucleotide sequences, including mtDNA sequences disclosed herein or fragments thereof, and their complements. As used herein, conditions of moderate stringency, as known to those having ordinary skill in the art, and as defined by Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed. Vol. 1, pp. 1.101-104, Cold Spring Harbor Laboratory Press (1989), include, for example, the use as a prewashing solution for nitrocellulose filters on which proband nucleic acids have been immobilized of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization conditions of 50% formamide, 6X SSC at 42°C (or other similar hybridization solution), and washing conditions of about 50-60°C, 0.5X SSC, 0.1% SDS. Conditions of high stringency are defined as hybridization conditions as above, and with washing at 60-68°C, 0.2X SSC, 0.1% SDS. In other embodiments, hybridization to an mtDNA nucleotide sequence may be at normal stringency, which is approximately 25-30°C below Tm of the native duplex (*e.g.*, 5X SSPE, 0.5% SDS, 5X Denhardt's solution, 50% formamide, at 42°C or equivalent conditions), at low stringency hybridizations, which utilize conditions approximately 40°C below Tm, or at high stringency hybridizations, which utilize conditions approximately 10°C below Tm. The skilled artisan will recognize that the temperature, salt concentration, and chaotrope composition of hybridization and wash solutions may be adjusted as necessary according to factors such as the length and nucleotide base composition of the probe. (*See also*, *e.g.*, Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing, 1987.) Thus, desired variations in stringency of hybridization conditions may be achieved by altering the time, temperature and/or concentration of the solutions used for prehybridization, hybridization and wash steps. Accordingly, it will be appreciated that suitably stringent conditions can be readily selected without undue experimentation where a desired selectivity of the probe is identified, based on its ability to hybridize to one or more certain proband sequences while not hybridizing to certain other proband sequences.

An "isolated nucleic acid molecule" refers to a polynucleotide molecule in the form of a separate fragment or as a component of a larger nucleic acid construct, that has been separated from its source cell (including the chromosome it normally resides in) at least once, preferably in a substantially pure form. Isolated nucleic acids may be nucleic acids having particular disclosed nucleotide sequences or may be regions, portions or fragments thereof. Those having ordinary skill in the art are able to prepare isolated nucleic acids having the complete nucleotide sequence, or the sequence of any portion of a particular isolated nucleic acid molecule, when provided with the appropriate nucleic acid sequence information as disclosed herein. Nucleic acid molecules may be comprised of a wide variety of nucleotides, including DNA, RNA, nucleotide analogues such as phosphorothioates or peptide nucleic acids, or other analogues with which those skilled in the art will be familiar, or some combination of these.

The present invention, as described herein, provides mtDNA sequences and isolated mtDNA nucleic acid molecules. mtDNA may be isolated from cellular DNA according to well known methodologies, for example those described in U.S. Patent No. 5,840,493, which is hereby incorporated by reference in its entirety.

Where it is advantageous to use oligonucleotide primers according to the present invention, such primers may be 10-60 nucleotides in length, preferably 15-35 nucleotides and still more preferably 18-30 nucleotides in length. Primers may be useful in the present invention for quantifying mtDNA mutations, including single nucleotide polymorphisms or homoplasmic mtDNA mutations provided herein, by any of a variety of techniques well known in the art for determining the amount of specific nucleic acid target sequences present in a sample based on specific hybridization of a primer to the target sequence. Optionally, in certain of these techniques, hybridization precedes nucleotide polymerase catalyzed extension of the primer using the strand containing the target sequence as a template, and/or ligation of oligonucleotides hybridized to adjacent target sequences, and embodiments of the invention using primer extension are particularly preferred.

For examples of references on such quantitative detection techniques, including those that may be used to detect nucleotide insertions, substitutions or deletions in a portion of an mtDNA sequence site near an oligonucleotide primer target hybridization site that corresponds to a portion of the wildtype mtDNA sequence as disclosed in Anderson et al. (1981 *Nature* 290:457, SEQ ID NO:1) or a mutated site such as may be created by any of the mtDNA point mutations disclosed herein, and further including those that involve primer extension, see U.S. 5,760,205 and the

references cited therein, all of which are hereby incorporated by reference, and see also, for example, Botstein et al. (*Am. J. Hum. Gen. 32*:314, 1980), Gibbs et al. (*Nucl. Ac. Res. 17*:2437, 1989), Newton et al. (*Nucl. Ac. Res. 17*:2503, 1989), Grossman et al. (*Nucl. Ac. Res. 22*:4527, 1994), and Saiki et al. (*Proc. Nat. Acad. Sci. 86*:6230, 1989), all of which are hereby incorporated by reference. A particularly useful method for this purpose is the primer extension assay disclosed by Fahy et al. (*Nucl. Acids Res. 25*:3102, 1997) and by Ghosh et al. (*Am. J. Hum. Genet. 58*:325, 1996), both of which references are hereby incorporated in their entireties, as is Krook et al. (*Hum. Molec. Genet. 1*:391, 1995) which teaches modification of primer extension reactions to detect multiple nucleotide substitutions, insertions, deletions or other mutations. Other examples of useful techniques for quantifying the presence of specific nucleic acid target sequences in a sample include but need not be limited to labeled probe hybridization to the target nucleic acid sequences with or without first partially separating target nucleic acids from other nucleic acids present in the sample.

Examples of other useful techniques for determining the amount of specific nucleic acid target sequences present in a sample based on specific hybridization of a primer to the target sequence include specific amplification of target nucleic acid sequences and quantification of amplification products, including but not limited to polymerase chain reaction (PCR, Gibbs et al., *Nucl. Ac. Res. 17*:2437, 1989), transcriptional amplification systems, strand displacement amplification and self-sustained sequence replication (3SR, Ghosh et al, in Molecular Methods for Virus Detection, 1995 Academic Press, NY, pp. 287-314), the cited references for which are hereby incorporated in their entireties. Examples of other useful techniques include ligase chain reaction, single stranded conformational polymorphism analysis, Q-beta replicase assay, restriction fragment length polymorphism (RFLP, Botstein et al., *Am. J. Hum. Gen. 32*:314, 1980) analysis and cycled probe technology, as well as other suitable methods that will be known to those familiar with the art.

In a particularly preferred embodiment of the invention, primer extension is used to quantify mtDNA mutations present in a biological sample. (Ghosh et al., *Am. J. Hum. Genet. 58*:325, 1996) This embodiment may offer certain advantages by permitting both wildtype and mutant mtDNA to be simultaneously quantified using a single oligonucleotide primer capable of hybridizing to a complementary nucleic acid target sequence that is present in a defined region of wildtype mtDNA and in a corresponding region of a mutated mtDNA sequence. Without wishing to be bound by theory, the use of a single primer for quantification of wildtype and mutated mtDNA is believed to avoid uncertainties associated with potential disparities in the relative

hybridization properties of multiple primers and may offer other advantages. Where such a target sequence is situated adjacent to a mutated mtDNA nucleotide sequence position that is a nucleotide substitution, insertion or deletion relative to the corresponding wildtype mtDNA sequence position, primer extension assays may be designed such that oligonucleotide extension products of primers hybridizing to mutated mtDNA are of different lengths than oligonucleotide extension products of primers hybridizing to wildtype mtDNA. Accordingly, the amount of mutant mtDNA in a sample and the amount of wildtype mtDNA in the sample may be determined by quantification of distinct extension products that are separable on the basis of sequence length or molecular mass.

Sequence length or molecular mass of primer extension assay products may be determined using any known method for characterizing the size of nucleic acid sequences with which those skilled in the art are familiar. In a preferred embodiment, primer extension products are characterized by gel electrophoresis. In another preferred embodiment, primer extension products are characterized by mass spectrometry (MS), which may further include matrix assisted laser desorption ionization/ time of flight (MALDI-TOF) analysis or other MS techniques known to those having skill in the art. See, for example, U.S. 5,622,824, U.S. 5,605,798 and U.S. 5,547,835, all of which are hereby incorporated by reference in their entireties. In another preferred embodiment, primer extension products are characterized by liquid or gas chromatography, which may further include high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS) or other well known chromatographic methodologies.

In another particularly preferred embodiment of the invention, DNA in a biological sample containing mtDNA is first amplified by methodologies well known in the art, such that the amplification products may be used as templates in a method for detecting single nucleotide polymorphisms or homoplasmic mtDNA mutations present in the sample. Accordingly, it may be desirable to employ oligonucleotide primers that are complementary to target sequences that are identical in, and common to, wildtype and mutant mtDNA, for example PCR amplification templates and primers prepared according to Fahy et al. (*Nucl. Acids Res.*, *25*:3102, 1997) and Davis et al. (*Proc. Nat. Acad. Sci. USA 94*:4526, 1997; *see also* Hirano et al., *Proc. Nat. Acad. Sci. USA 94*:14894, 1997, and Wallace et al., *Proc. Nat. Acad. Sci. USA 94*:14900, 1997.)

In certain other preferred embodiments, mtDNA mutations may be efficiently detected, screened and/or quantified by high throughput hybridization methodologies directed to independently probing a plurality of distinct mtDNAs, or a

plurality of distinct oligonucleotide primers as provided herein, that have been immobilized as nucleic acid arrays on a solid phase support. Typically, the solid support may be silica, quartz or glass, or any other material on which nucleic acid may be immobilized in a manner that permits appropriate hybridization, washing and detection steps as known in the art and as provided herein. In preferred embodiments, solid-phase nucleic acid arrays are precisely spatially addressed, as described, for example, U.S. Patent 5,800,992 (see also, *e.g.*, WO 95/21944; Schena et al., 1995 *Science* 270:467-470, 1995; Pease et al., *1994 Proc. Nat. Acad. Sci. USA* 91:5022; Lipshutz et al., 1995 *Biotechniques 19*: 442-447).

Detection of hybridized (*e.g.*, duplexed) nucleic acids on the nucleic acid array may be achieved according to any known procedure, for example, by spectrometry or potentiometry (*e.g.*, MALDI-MS). Within certain preferred embodiments the array contains oligonucleotides that are less than 50 bp in length. For high throughput screening of nucleic acid arrays, the format is preferably amenable to automation. It is preferred, for example, that an automated apparatus for use according to high throughput screening embodiments of the present invention is under the control of a computer or other programmable controller. The controller can continuously monitor the results of each step of the nucleic acid deposition, washing, hybridization, detection and related processes, and can automatically alter the testing paradigm in response to those results.

The present invention also provides compositions and methods that are useful in pharmacogenomics, for the classification and/or stratification of a subject or a patient population. Such stratification may involve, for example, correlation of single nucleotide polymorphisms or homoplasmic mtDNA mutations as provided herein with, for instance, one or more particular traits in a subject, and further, optionally, with indicators of the responsiveness to, or efficacy of, a particular therapeutic treatment. In one aspect of the invention, detection in a biological sample of single nucleotide polymorphisms or homoplasmic mtDNA mutations that segregate with AD from a subject is combined with identification of the subject's apolipoprotein E (APOE) genotype to determine the risk for, or presence of, Alzheimer's disease (AD) in the subject. The apolipoprotein E type 4 allele (*APOE*-ε4) allele is a genetic susceptibility factor for sporadic AD and confers a two fold risk for AD (Corder et al., *Science 261*:921, 1993; *see also* "National Institute on Aging/Alzheimer's Association Working Group Consensus Statement," *Lancet 347*:1091, 1996 and references cited therein, all of which are hereby incorporated by reference in their entireties.). Accordingly, in a preferred embodiment of the invention, the method for determining the risk for or

presence of AD in a subject by detecting single nucleotide polymorphisms or homoplasmic mtDNA point mutations according to the present disclosure will further comprise determining the APOE genotype of the subject suspected of being at risk for AD. By using the combination of the methods for determining mtDNA point mutations as disclosed herein, and methods known in the art for determining APOE genotype, an enhanced ability to detect the relative risk for AD is provided by the instant invention along with other related advantages. Similarly, where APOE genotype and risk for AD are correlated, the present invention provides advantageous methods for identifying agents suitable for treating AD where such agents affect may be correlated with detection of one or more specific single nucleotide polymorphisms or homoplasmic mtDNA mutations in a biological source.

As described herein, determination of specific single nucleotide polymorphisms or homoplasmic mtDNA mutations may be used to stratify an AD patient population. Accordingly, in another preferred embodiment of the invention, determination of such mutations in a biological sample from an AD subject may provide a useful correlative indicator for that subject. An AD subject so classified on the basis of one or more specific mutations may then be monitored using AD clinical parameters referred to above, such that correlation between particular mtDNA mutations and any particular clinical score used to evaluate AD may be monitored. For example, stratification of an AD patient population according to at least one of the single nucleotide polymorphisms or homoplasmic mtDNA mutations provided herein may provide a useful marker with which to correlate the efficacy of any candidate therapeutic agent being used in AD subjects. In a further preferred embodiment of this aspect of the invention, determination of one or more specific mtDNA mutations in concert with determination of an AD subject's APOE genotype, as described above, may also be useful. These and related advantages will be appreciated by those familiar with the art.

In particularly preferred embodiments, oligonucleotide primers will be employed that permit specific detection of the single nucleotide polymorphisms or homoplasmic mtDNA point mutations disclosed in Tables 1-4 and Table 9, wherein specific substitution and deletion mutations in mitochondrial genes including, for example, those encoding 12S rRNA, 16S rRNA, several tRNAs, COX1, COX2, COX3, cytochrome b, ATPase 8, ATPase 6, ND1, ND2, ND4 and ND5 are disclosed, as are numerous mutations in the mtDNA D-loop region. Each mutation listed in Tables 1-4 and Table 9 is designated with (i) the identity of the nucleotide at a particular nucleotide position according to the wildtype human mtDNA sequence (Anderson et al., 1981

Nature 290:457; *see also* Andrews et al., 1999 *Nature Genetics* 23:147 and references cited therein), (ii) the nucleotide position number according to the convention of Anderson et al. (1981) and (iii) the identity of the mutated nucleotide at that position, identified as disclosed herein. Thus, for example, the nucleotide T (thymine) situated at position 1189 of the wildtype mtDNA 12S rRNA gene is mutated to the nucleotide C (cytosine) in mtDNA analyzed from a substantial number of patients diagnosed with AD (see Tables 1, 2, 4). Table 9 also presents the number of samples in which a particular AD-associated SNP was detected, and the mitochondrial haplogroup of the mtDNA sample donor in which the AD-associated SNP was first identified (see also Examples, below).

As presented in Tables 2-4 and as disclosed herein, a mitochondrial single nucleotide polymorphism or homoplasmic mtDNA point mutation, which includes a deviation in the identity of the nucleotide base situated at a specific position in a mtDNA sequence relative to the "wildtype" human mtDNA sequence (CRS) disclosed by Anderson et al. (1981), may fall into at least one of the following categories: An "error" refers to sequencing mistakes in the human mtDNA sequence reported by Anderson et al. (1981), as corrected by Andrews et al. (1999 *Nature Genetics* 23:147). A "polymorphism" in Tables 2-4 refers to a known polymorphism in a human mtDNA sequence that is not associated with a particular human disease, but that has been detected and described as a result of naturally occurring variability in the identity of the nucleotide base situated at a given position in a human mtDNA sequence (see, *e.g.*, "Mitomap", Emory University School of Medicine, available at http://www.gen.emory.edu). A "rare polymorphism" in Tables 2-4 refers to a mtDNA nucleotide that differs from the base situated at the corresponding position in the Cambridge Reference Sequence (CRS) of Anderson et al. (1981) but which, upon subsequent accumulation of human mtDNA sequence data from a plurality of subjects (and in contrast to the reliance of Anderson et al. upon the mtDNA sequence of a single donor to generate the CRS), suggests the presence of a low frequency allele in the CRS donor, relative to the larger sample population (see Andrews et al., 1999 *Nature Genetics* 23:147 and references cited therein). Particularly useful mutations that segregate with AD according to the present invention include homoplasmic mtDNA point mutations (*e.g.*, single nucleotide polymorphisms) presented in Tables 2-4 that are not errors, polymorphisms or rare polymorphisms as just described, and additionally, the homoplasmic mtDNA point mutations (*e.g.*, single nucleotide polymorphisms) presented in Table 9.

Table 1

HOMOPLASMIC MITOCHONDRIAL MUTATIONS IN RIBOSOMAL RNA GENES

PATIENT	TISSUE	DIAGNOSIS	12S rRNA					
SY5Y	Parental cells	C						
3806	Blood	C						
3862	Blood	C						
4157	Blood	C					T1189C	
4158	Blood	C						
4175	Blood	C						
4176	Blood	C	G709A					
4253	Blood	C					T1189C	
4313	Blood	C						
4314	Blood	C					T1189C	
4380	Blood	C						
369	Blood	C (AC/ADRC)						
3B4_WE	Brain (FC)	NC						
8B5_KI	Brain (FC)	PSP						
8A4_KO	Brain (FC)	PSP	G709A					
8C4_SC	Brain (FC)	DAG						
8A1_QU	Brain (FC)	Picks						
8A2_GO	Brain (FC)	Wallerian						
8A3_OO	Brain (FC)	Hallervorden						

PATIENT	TISSUE	DIAGNOSIS	12S rRNA					
8B1_UN	Brain (FC)	HD	G709A					
8B3_PO	Brain (FC)	DLBD						
8B6_SI	Brain (FC)	DLBD						
8C1_LL	Brain (FC)	DLBD					T1189C	
8C2_KK	Brain (FC)	DLBD						
8C3_IA	Brain (FC)	DLBD	G709A					
1685 B1+Cy	Blood/Cybrid	AD					T1189C	
3219 B1	Blood/Cybrid	AD				T980C		
3807	Blood	AD						
3810	Blood	AD					T1189C	
3812	Blood	AD						
3813	Blood	AD						
3814	Blood	AD						
3815	Blood	AD					T1189C	
3816	Blood	AD						
3818	Blood	AD						
3819	Blood	AD						
3822	Blood	AD					T1189C	
3823	Blood	AD						
381	Blood	AD (AC/FB)						
383	Blood	AD (AC/FB)					T1189C	
384	Blood	AD (AC/FB)						

PATIENT	TISSUE	DIAGNOSIS	12S rRNA					
474	Blood	AD (AC/FB)	G709A					T1243C
478	Blood	AD (AC/FB)					T1189C	
973	Blood	AD (AC/ADRC)						
1244	Blood	AD (AC/ADRC)						
1461	Blood	AD (AC/ADRC)						
1517	Blood	AD (AC/ADRC)						
2552	Blood	AD (AC/ADRC)					T1189C	
2693	Blood	AD (AC/ADRC)						
8C5_MC	Brain (FC)	LBV					T1189C	
8C6_NI	Brain (FC)	LBV					T1189C	
8C7_MO	Brain (FC)	LBV						
8C8_RS	Brain (FC)	LBV	G709A	G930A				
8D1_BS	Brain (FC)	LBV						
8D2_MD*	Brain (FC)	AD			960DEL			
8D3_LC	Brain (FC)	AD						
8D4_WI	Brain (FC)	AD						
8D5_JE	Brain (FC)	AD	G709A					
8D6_DE*	Brain (FC)	AD	G709A					
3A5_YA	Brain (FC)	AD					T1189C	
3A6_BR	Brain (FC)	AD	G709A	G930A				
8A7_SA	Brain (FC)	AD						
8A8_BA	Brain (FC)	AD						
8B2_SP	Brain (FC)	AD	G709A					

PATIENT	TISSUE	DIAGNOSIS	16S rRNA										
SY5Y	Parental cells	C											
3806	Blood	C										G3010A	
3862	Blood	C										G3010A	
4157	Blood	C				A1811G							
4158	Blood	C											
4175	Blood	C											
4176	Blood	C				A1811G		G2098A					
4253	Blood	C				A1811G							
4313	Blood	C											
4314	Blood	C				A1811G							
4380	Blood	C											
369	Blood	C (AC/ADRC)											
3B4_WE	Brain (FC)	NC											
8B5_KI	Brain (FC)	PSP											
8A4_KO	Brain (FC)	PSP					G1888A						
8C4_SC	Brain (FC)	DAG											
8A1_QU	Brain (FC)	Picks				A1811G							

PATIENT	TISSUE	DIAGNOSIS	16S rRNA										
8A2_GO	Brain (FC)	Wallerian											
8A3_OO	Brain (FC)	Hallervorden											
8B1_UN	Brain (FC)	HD					G1888A						
8B3_PO	Brain (FC)	DLBD										G3010A	
8B6_SI	Brain (FC)	DLBD											
8C1_LL	Brain (FC)	DLBD											
8C2_KK	Brain (FC)	DLBD											
8C3_IA	Brain (FC)	DLBD				A1811G							
1685 Bl+Cy	Blood/ Cybrid	AD				A1811G							
3219 Bl	Blood/ Cybrid	AD											
3807	Blood	AD											
3810	Blood	AD				A1811G							
3812	Blood	AD											
3813	Blood	AD							T2158C				
3814	Blood	AD											

PATIENT	TISSUE	DIAGNOSIS	16S rRNA										
3815	Blood	AD											
3816	Blood	AD											T3197C
3818	Blood	AD	C1700T										
3819	Blood	AD										G3010A	
3822	Blood	AD				A1811G							
3823	Blood	AD										G3010A	
381	Blood	AD (AC/FB)										G3010A	
383	Blood	AD (AC/FB)				A1811G							
384	Blood	AD (AC/FB)											
474	Blood	AD (AC/FB)											
478	Blood	AD (AC/FB)				A1811G							
973	Blood	AD (AC/ADRC)											T3197C
1244	Blood	AD (AC/ADRC)								C2259T			
1461	Blood	AD (AC/ADRC)											
1517	Blood	AD (AC/ADRC)											
2552	Blood	AD (AC/ADRC)				A1811G							
2693	Blood	AD (AC/ADRC)		G1719A									
8C5_MC	Brain (FC)	LBV				A1811G							
8C6_NI	Brain (FC)	LBV				A1811G							
8C7_MO	Brain (FC)	LBV											

PATIENT	TISSUE	DIAGNOSIS	16S rRNA										
8C8_RS	Brain (FC)	LBV					G1888A						
8D1_BS	Brain (FC)	LBV										G3010A	
8D2_MD*	Brain (FC)	AD			T1809C								
8D3_LC	Brain (FC)	AD										G3010A	
8D4_WI	Brain (FC)	AD									T2352C	G3010A	
8D5_JE	Brain (FC)	AD											
8D6_DE*	Brain (FC)	AD					G1888A						
3A5_YA	Brain (FC)	AD				A1811G							
3A6_BR	Brain (FC)	AD					G1888A						
8A7_SA	Brain (FC)	AD											
8A8_BA	Brain (FC)	AD										G3010A	
8B2_SP	Brain (FC)	AD											

Key: AC, autopsy control; ADRC, Alzheimer's Disease Research Center (San Diego, CA); C, control; DAG, dementia with argyrophilic grains; DLBD, diffuse Lewy body, dementia; FB, M. F. Beal, Cornell Univ. Med. Ctr. (NY); HD, Huntington's disease; LBV, Lewy body variant; NC, normal control (same as "control"); PSP, parasupranuclear palsy.

Table 2

SEQUENCE ANALYSIS OF AD-1 HUMAN MITOCHONDRIAL DNA

Nucleotide position	Gene	Mutation	CRS	AD-1	Remarks
73	D-Loop	-	A	G	Polymorphism[2]
114	D-Loop	-	C	T	Polymorphism[2]
263	D-Loop	-	A	G	rare polymorphism in CRS[1]
311-315	D-Loop	-	CTC	CCTCC	rare polymorphism in CRS[1]
750	12S rRNA	-	A	G	rare polymorphism in CRS[1]
1189	12S rRNA	-	T	C	
1438	12S rRNA	-	A	G	rare polymorphism in CRS[1]
1811	16S rRNA	-	A	G	Polymorphism[2]
2706	16S rRNA	-	A	G	Polymorphism[2]
3106-3107	16S rRNA	-	CC	C	Error[1]
3423	ND1	-	G	T	Error[1]
3480	ND1	-	A	G	Polymorphism[2]
4769	ND2	-	A	G	rare polymorphism in CRS[1]
4985	ND2	-	G	A	Error[1]
6366	COI	Val155Ile	G	A	
7028	COI	-	C	T	Polymorphism[2]
8860	ATPase 6	Thr112Ala	A	G	rare polymorphism in CRS[1]
9055	ATPase 6	Ala177Thr	G	A	polymorphism[2]
9559	COIII	Ala118Pro	G	C	error[1]
10398	ND3	Thr114Ala	A	G	polymorphism[2]
10550	ND4L	-	A	G	polymorphism[2]
11299	ND4	-	T	C	polymorphism[2]
11335	ND4	-	T	C	error[1]
11467	ND4	-	A	G	polymorphism[2]
11719	ND4	-	G	A	polymorphism[2]
11914	ND4	-	G	A	polymorphism[2]
12308	ND5	-	A	G	polymorphism[2]
12372	ND5	-	G	A	polymorphism[2]
12954	ND5	-	T	C	
13702	ND5	Gly456Arg	G	C	error[1]
14167	ND6	-	C	T	polymorphism[2]
14199	ND6	-	G	T	error[1]
14272	ND6	-	G	C	error[1]
14365	ND6	-	G	C	error[1]
14368	ND6	-	G	C	error[1]

Nucleotide position	Gene	Mutation	CRS	AD-1	Remarks
15326	Cytb	Thr194Ala	A	G	rare polymorphism in CRS[1]
15924	$tRNA^{Thr}$	-	A	G	polymorphism[2]
16519	D-Loop	-	T	C	polymorphism[2]

1 Andrews et al. (1999) *Nature Gen. 23.* 147
2 Mitomap. Emory University School of Medicine (www.gen.emory.edu)

Table 3

SEQUENCE ANALYSIS OF AD-2 HUMAN MITOCHONDRIAL DNA

Nucleotide position	Gene	Mutation	CRS	AD-2	Remarks
263	D-Loop	-	A	G	rare polymorphism in CRS[1]
311-315	D-Loop	-	CTC	CCTCC	rare polymorphism in CRS[1]
750	12S rRNA	-	A	G	rare polymorphism in CRS[1]
980	12S rRNA	-	T	C	
3010	16S rRNA	-	G	A	polymorphism[2]
3106-3107	16S rRNA	-	CC	C	error[1]
3423	ND1	-	G	T	error[1]
4769	ND2	-	A	G	rare polymorphism in CRS[1]
4985	ND2	-	G	A	error[1]
8860	ATPase 6	Thr112Ala	A	G	rare polymorphism in CRS[1]
9559	COIII	Ala118Pro	G	C	error[1]
11335	ND4	-	T	C	error[1]
13702	ND5	Gly456Arg	G	C	error[1]
14199	ND6	-	G	T	error[1]
14272	ND6	-	G	C	error[1]
14365	ND6	-	G	C	error[1]
14368	ND6	-	G	C	error[1]
14766	Cytb	Ile7Thr	T	C	polymorphism
15326	Cytb	Thr194Ala	A	G	rare polymorphism in CRS[1]
15924	$tRNA^{Thr}$	-	A	G	polymorphism[2]
16519	D-Loop	-	T	C	polymorphism[2]

1 Andrews et al. (1999) *Nature Gen. 23.* 147
2 Mitomap. Emory University School of Medicine (www.gen.emory.edu)

Table 4

SNP'S IN THE MITOCHONDRIAL rRNA GENES

Patient Diagnosis	N	Nucl. Pos.	# Patients	Remarks	
		A750G	111 of 112	rare polymorphism in CRS[1]	
		A1438G	108 of 112	rare polymorphism in CRS[1]	
		A2706G	65 of 112	polymorphism[2]	
		C3107Del	111 of 112	error in CRS[1]	
Living Controls	N=13	G709A	1 of 13	polymorphism[2]	hapologroup T, some W
		T1189C	4 of 13		
		A1811G	5 of 13	polymorphism[2]	haplogroup U
		G2098A	1 of 13		
		G3010A	3 of 13	polymorphism[2]	haplogroup H, some Y
Autopsy-Confirmed Controls	N=41	G709A	6 of 41	polymorphism[2]	haplogroup T, some W
		T789C	1 of 41		
		793T ins	1 of 41		
		C870T	1 of 41		
		G930A	1 of 41		
		T980C	1 of 41		
		T1189C	3 of 41		
		T1243C	1 of 41		
		G1719A	1 of 41	polymorphism[2]	
		T1809C	1 of 41		
		A1811G	5 of 41	polymorphism[2]	haplogroup U
		G1888A	4 of 41		haplogroup T
		A2294G	1 of 41		
		A2581G	1 of 41		
		G3010A	5 of 41	polymorphism[2]	haplogroup H, some Y

Patient Diagnosis	N	Nucl. Pos.	# Patients	Remarks	
Living ADs	N=13	T980C	1 of 13		
		T1189C	4 of 13		
		C1700T	1 of 13		
		A1811G	3 of 13	polymorphism[2]	haplogroup U
		T2156C	1 of 13		
		T2158C	1 of 13		
		G30110A	2 of 13	polymorphism[2]	haplogroup H, some Y
		T3197C	1 of 13	polymorphism[2]	
Autopsy-Confirmed ADs	N=45	T669C	1 of 45		
		G709A	8 of 45	polymorphism[2]	haplogroup T, some W
		G930A	2 of 45	polymorphism[2]	haplogroup T
		C960Del	1 of 45		
		G1007A	1 of 45		
		T1189C	9 of 45		
		T1243C	1 of 45		
		G1393A	1 of 45		
		G1709A	1 of 45		
		G1719A	3 of 45	polymorphism[2]	
		A1811G	9 of 45	polymorphism[2]	haplogroup U
		G1888A	5 of 45		haplogrooup T
		C2259T	1 of 45		
		T2352C	1 of 45	polymorphism[2]	
		T2483C	1 of 45		
		A2851G	1 of 45		
		G3010A	10 of 45	polymorphism[2]	haplogroup H, some Y
		T3197C	2 of 45	polymorphism[2]	

[1] Andrews et al. (1999) *Nature Gen. 23*, 147.
[2] Mitomap, Emory University School of Medicine (www.gen.emory.edu)

The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

EXAMPLE 1

DNA ISOLATION FROM BLOOD AND BRAIN SAMPLES

Venous blood samples were obtained from donor individuals and collected in vacutainer tubes containing EDTA. White blood cell fractions were obtained by centrifugation at 2,500 rpm at 4°C for 30 min. The white blood cell layers were collected and diluted with 5 ml sterile TE buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA) and centrifuged at 2,500 rpm for 10 min at 4°C. The cell pellet was then lysed by the addition of 10 ml lysis buffer containing 1% SDS and 400 µg/ml proteinase K in TE buffer. Cells were incubated for 4 hrs at 37°C while shaking at 200 rpm in an orbital shaker. Total cellular DNA was purified by two extractions with phenol/chloroform and two extractions with chloroform. DNA was precipitated by adding 1/10 volume 5 M NaCl and 2x volume 100% ethanol and placed at –20°C. DNA was pelleted by centrifugation, washed with 70% ethanol, and resuspended in TE buffer.

Total cellular DNA was also isolated from frozen brain tissue by homogenizing the tissue in lysis buffer (50 mM Tris-HCl, pH 7.9, 100 mM EDTA, 0.1 M NaCl, 0.03 M DDT, 1% SDS, 1 mg/ml proteinase K) using a dounce glass homogenizer. The homogenized brain tissue was incubated for 30-60 min at 45-50°C. DNA was purified by two extractions with phenol/chloroform and two extractions with chloroform. DNA was precipitated by adding 1/10 volume 5 M NaCl and 2x volume 100% ethanol and placed at –20°C. DNA was pelleted by centrifugation, washed with 70% ethanol, and resuspended in TE buffer.

Total cellular DNA was also isolated from human SH-SY5Y neuroblastoma cells by first removing the cells from the tissue culture flasks by treatment with 0.5 mM EDTA in Dulbecco's PBS. Cells were then pelleted by centrifugation at 2,000 g for 10 min and DNA was extracted with DNAzol reagent (Molecular Research Center, Inc., Cincinnati, OH) according to the manufacturer's recommendations.

DNA concentrations were determined by UV absorption at 260 nm.

EXAMPLE 2

PCR AMPLIFICATION, SEQUENCING, AND SEQUENCE ANALYSIS

Total cellular DNA prepared from white blood cells, brain tissue, and SH-SY5Y cells, as described in Example 1, was used for amplification with sets of oligonucleotide primers specific for the indicated regions of mtDNA light strand (Table 5, "L" primers) and heavy strand (Table 5, "H") nucleotide sequences, or primers complementary to portions of mtDNA D-loop regions (see, *e.g.*, Wallace et al., in *Mitochondria & Free Radicals in Neurodegenerative Diseases*, M.F. Beal, N. Howell and I. Bodis-Wollner, eds., 1997 Wiley-Liss, Inc., pp. 283-307, and references cited therein) that are capable of amplifying a region spanning essentially the entire mitochondrial DNA molecule (Table 6). Amplifications were performed in 50 µl reaction volumes using 0.5-1.0 µg of DNA, 200 ng each of L-strand and H-strand or forward (Table 6, "F") and reverse (Table 6, "R") primers, 200 µM of each dNTP, 10 mM Tris-HCl, pH 8.3, 50 mM KCL, 2 mM $MgCl_2$, and one unit of AmpliTaq DNA polymerase (Perkin-Elmer, Norwalk, CT). Using a Gene Amp PCR System 9600 thermal cycler (Perkin-Elmer), amplification was carried out as follows: 95°C for 10 sec, 30 cycles at 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min. and 1 cycle at 72°C for 4 min. Amplicons were purified by the QIAquick PCR Purification Kit (Qiagen, Chatsworth, CA).

Table 5

PRIMERS SPECIFIC FOR INDICATED REGIONSOF MITOCHONDRIAL GENOME

Fragment #	Nucleotide	Sequence 5`-3`	Primer Length	Td	Region Covered	Fragment Length	SEQ ID NO:
110	50IL	CCATCCTACCCAGCAC ACA	19	63.7	12S	362	
110	837H	TATAGCTTAGTTAAAC TTTCGTTTAT	26	59	12S	362	
111	770L	CAATGCAGCTCAAAAC GC	18	62.5	12S	378	
111	1130H	CGTAGTGTTCTGGCGA GC	18	61.3	12S	378	
112	1065L	CCAAACTGGGATTAGA TACCC	21	62.3	12S	385	
112	1424H	CACTCTACTCTTAGTTT ACTGCTAAA	26	59.3	12S	385	
113	1372L	CTACCCCAGAAAACTA CGATAG	22	60.1	12S/16S	365	
113	1719H	TTGGGTAAATGGTTTG GC	18	60.7	12S/16S	365	
114	1649L	CAACTTAACTTGACCG CTCTG	21	62.2	16S	368	
114	2000H	GCTATCACCAGGCTCG G	17	60.8	16S	368	

Fragment #	Nucleotide	Sequence 5'-3'	Primer Length	Td	Region Covered	Fragment Length	SEQ ID NO:
115	1934L	TAAAAGAGCACACCCG TCTA	20	60.3	16S	367	
115	2305H	AGGAGAATGTTTTCAT GTTACTTATA	26	61	16S	397	
116	2255L	CTCACACCCAATTGGA CC	18	60	16S	369	
116	2602H	TCCCTATTTAAGGAAC AAGTGA	22	60.7	16S	369	
117	2544L	CTGCCCAGTGACACAT GTT	19	60.9	16S	386	
117	2911H	GGGTAACTTGTTCCGT TGG	19	62	16S	386	
118	2861L	ACTTCACCAGTCAAAG CGA	19	60.3	16S	333	
118	3171H	AGTTGAGATGATATCA TTTACGG	23	60.2	16S	333	
119	3091L	GTCGGTTTCTATCTAC CTTCAA	22	60.2	16S	301	
119	3370H	CTAGAATTTTTCGTTC GGTAAG	22	60.7	16S	301	

Table 6

D-LOOP PCR PRIMERS

Primer	Nucleotide	Sequence 5'-> 3'	False Priming	SEQ ID NO:
51F	15968	TCTTTAACTCCACCATT AGCAC		
51R	16388	AGGATGGTGGTCAAGG GACCCC		
52F	16343	ACAGTCAAATCCCTTC TCGTCC		
52R	241	GGCTGTGCAGACATTC AATTGT		
53F	165	ACCTACGTTCAATATT ACAGGC		
53R	598	AACATTTTCAGTGTATT GCTTT		

Sequencing of the purified PCR products was performed using the same primer as used previously for amplification and the BigDye Terminator Cycle Sequencing kit (Perkin-Elmer). Sequencing reaction products were purified by ethanol precipitation or with CentriSep spin columns (Princeton Separations, Adelphia, NJ) and electrophoresed in the Applied Biosystems Model 373A DNA sequencing system (Applied Biosystems Division, Perkin-Elmer, Foster City, CA). The Sequence Navigator software (Applied Biosystems Division, Perkin-Elmer) was used for analysis of sequence data, in addition to CAP and ALIGN sequence analysis programs, and mutations were identified by comparison with the published sequence of human mtDNA (Anderson et al., 1981 *Nature* 290:457).

EXAMPLE 3

SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN MITOCHONDRIAL RIBOSOMAL RNAS THAT SEGREGATE WITH ALZHEIMER'S DISEASE

Mitochondrial DNA (mtDNA) was isolated and sequenced from brain and blood samples of 24 controls (12 autopsy-confirmed disease controls, 2 autopsy-confirmed normal controls, and 10 living controls) and 33 AD cases (20 autopsy-confirmed AD, and 13 living AD patients) according to Examples 1 and 2. Altered mitochondrial rRNA sequences were detected in 82% of AD samples versus 50% of controls (Table 1 and Figure 1). When this analysis was restricted to only autopsy-

confirmed (AC) cases, rRNA sequence changes were detected in 85% of AD and 43% of control samples.

Approximately half of the mtDNA changes were associated with haplogroups T, U, and H (familial lineages). Thus, these haplogroups did not strictly track with AD. However, considering only mutations in mtDNA encoding mitochondrial rRNA that are not associated with haplogroups, 49% of AD versus 21% of control samples carried sequence changes. (Table 1 and Figure 1). The specific correlation of AD with non-haplogroup associated mutations in mtDNA encoding mitochondrial rRNA was higher when only autopsy-confirmed cases were included. Mutations in mtDNA encoding mitochondrial rRNA are detected in 40% of AD versus 7% of autopsy-confirmed control cases.

EXAMPLE 4

HOMOPLASMIC SINGLE NUCLEOTIDE POLYMORPHISMS IN THE MITOCHONDRIAL DNA OF A SUBJECT DIAGNOSED WITH ALZHEIMER'S DISEASE

This example describes detection of mtDNA single nucleotide polymorphisms in DNA obtained from white blood cells of a subject (D-1) diagnosed as having Alzheimer's Disease, and in a cybrid cell line prepared using platelet-derived mtDNA from the same subject.

Venous blood samples were collected from a human donor subject (D-1) diagnosed with AD (McKhann et al., *Neurology 34*:939, 1984, National Institute of Neurology, Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association Criteria of Probable AD, NINCDS-ADRDA) into vacutainer tubes (Becton-Dickinson, Inc., San Jose, CA) containing EDTA and maintained at 0-4°C for preparation of white blood cell fractions, or containing acid citrate/ dextrose and maintained at ambient room temperature for preparation of platelet fractions.

To prepare white blood cell fractions, the blood samples were layered onto Histopaque®-1077 (Sigma, St. Louis, MO) and centrifuged at 2500 rpm for 30 min at 4°C. The white blood cell layers were collected and diluted with 5 ml sterile TE buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA) and centrifuged at 2500 rpm for 10 min at 4°C. The cell pellet was then lysed by the addition of 10 ml lysis buffer (TE containing 1% SDS and 400 µg/ml proteinase K, both from Sigma) and incubated for 4 hrs at 37 °C in an orbital shaker set at 200 rpm. Total cellular DNA was purified by two extractions with phenol/chloroform and two extractions with chloroform. DNA was precipitated by adding 1/10 volume 5 M NaCl and 2x volume 100% ethanol, and

incubating at -20°C. DNA was pelleted by centrifugation, washed with 70% ethanol and resuspended in TE buffer.

Preparation of platelets and use of the platelets (as a source of mtDNA) for fusion to ρ^0 SH-SY5Y neuroblastoma cells to produce a cybrid cell line was as described by Miller et al. (1996 *J. Neurochem.* 67:1897-1907). The resulting cybrid cell line (AD-1) had a stable deficiency in mitochondrial Complex IV (cytochrome c oxidase, COX, CO) activity (described below). Expanded cultures of AD-1 cybrid cells were dislodged from tissue culture flasks by exposing them to 0.5 mM EDTA in Dulbecco's PBS (GIBCO-BRL, Grand Island, NY). Harvested AD-1 cells were pelleted by centrifugation (2000 x *g*, 10 min) and total cellular DNA was extracted from the cell pellet with DNAzol reagent (Molecular Research Center, Inc., Cincinnati, OH) according to the manufacturer's instructions.

DNA concentrations were determined by UV absorption at 260 nm. Total cellular DNA prepared from AD white blood cells and from AD cybrid cells was used as template for DNA amplification by polymerase chain reaction (PCR) with sets of L-strand and H-strand oligonucleotide primers spanning the entire mtDNA molecule (Table 7). Amplifications were performed in 50 µl reaction volumes using 0.5-1.0 µg of template DNA, 200 ng each of L-strand and H-strand or forward and reverse primers, 200 µM of each dNTP, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 MM $MgCl_2$ and one unit of AmpliTaq DNA polymerase (Perkin-Elmer, Inc., Norwalk, CT). Amplification was carried out as follows using a GeneAmp™ PCR System 9600 thermal cycler (Perkin-Elmer): 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min. for 30 cycles followed by one cycle at 72°C for 4 min. Amplification products were purified by horizontal agarose gel electrophoresis, band excision, elution of DNA from the agarose, and ethanol precipitation. Alternatively, amplicons were purified by the QIAquick™ PCR purification kit (Qiagen, Chatsworth, CA).

PCR products were cloned using the TA-Cloning™ kit (Invitrogen, Inc., Carlsbad, CA) and XL2Blue™ or XL2BlueMRF™ competent cells (Stratagene, Inc., La Jolla, CA), all according to the suppliers' recommendations. Recombinant colonies were selected and plasmid DNA was purified using the Wizard™ Series 9600 DNA purification system (Promega, Inc., Madison, WI).

Table 7

PCR PRIMERS FOR MTDNA

FRAG	PRIMER	GENE	NUCLEOTIDE	PRIM. LENGTH	PRIMER SEQUENCE 5'- >3'	FRAG. LENGTH	SEQ ID NO:
0							
1	52F	D-LOOP	16343	22	ACAGTCAAATCCCTTCTCGTCC	489	
	52R	D-LOOP	241	22	GGCTGTGCAGACATTCAATTGT	489	
2	53F	D-LOOP	165	22	ACCTACGTTCAATATTACAGGC	455	
	53R	D-LOOP	598	22	AACATTTTCAGTGTATTGCTTT	455	
3	110F	TRNA Phe/12S	501L	19	CCATCCTACCCAGCACACA	362	
	110R	TRNA Phe/12s	837H	26	TATAGCTTAGTTAAACTTTCGTTTAT	362	
4	111F	12S	770L	18	CAATGCAGCTCAAAACGC	378	
	111R	12S	1130H	18	CGTAGTGTTCTGGCGAGC	378	
5	112F	12S	1065L	21	CCAAACTGGGATTAGATACCC	385	
	112R	12S	1424H	26	CACTCTACTCTTAGTTTACTGCTAAA	385	
6	113F	12S/tRNA Val/16S	1372L	22	CTACCCCAGAAAACTACGATAG	365	
	113R	12S/tRNA Val/16S	1719H	18	TTGGGTAAATGGTTTGGC	365	
7	114F	16S	1649L	21	CAACTTAACTTGACCGCTCTG	368	
	114R	16S	2000H	17	GCTATCACCAGGCTCGG	368	
8	115F	16S	1934L	20	TAAAAGAGCACACCCGTCTA	367	
	115R	16S	2305H	26	AGGAGAATGTTTTCATGTTACTTATA	367	
9	116F	16S	2255L	18	CTCACACCCAATTGGACC	369	
	116R	16S	2602H	22	TCCCTATTTAAGGAACAAGTGA	369	
10	117F	16S	2544L	19	CTGCCCAGTGACACATGTT	386	
	117R	16S	2911H	19	GGGTAACTTGTTCCGTTGG	386	
11	118F	16S	2861L	19	ACTTCACCAGTCAAAGCGA	333	
	118R	16S	3171H	23	AGTTGAGATGATATCATTTACGG	333	
12	119F	16S/tRNA Leu	3091L	22	GTCGGTTTCTATCTACCTTCAA	301	
	119R	16S/tRNA Leu	3370H	22	CTAGAATTTTTCGTTCGGTAAG	301	
13	61F	ND1	3281	23	GAGGTTCAATTCCTCTTCTTAAC	372	
	61R	ND1	3631	22	TTGAGTAAACGGCTAGGCTAGA	372	
14	62F	ND1	3589	20	CTGGTCAACCTCAACCTAGG	373	
	62R	ND1	3946	16	GGCCTGCGGCGTATTC	373	
15	63F	ND1	3908	17	CCGAAGGGGAGTCCGAA	387	
	63R	ND1	4270	25	ATCAAAGTAACTCTTTTATCAGACA	387	
16	120F	TrnAs Ile/Gln/Met	4186L	22	CTACCACTCACCCTAGCATTAC	370	
	120R	TRNAs Ile/Gln/Met	4539H	20	GTAAAAAATCAGTGCGAGCT	370	
17	71F	ND2	4447	20	TTGGTTATACCCTTCCCGTA	348	
	71R	ND2	4769	26	CTATTCCTAGTTTTATTGCTATAGCT	348	
18	72F	ND2	4699	22	ACAATATACTCTCCGGACAATG	378	
	72R	ND2	5054	23	GAATGGTTATGTTAGGGTTGTAC	378	
19	73F	ND2	4990	22	AGCTACGCAAAATCTTAGCATA	340	

FRAG	PRIMER	GENE	NUCLEOTIDE	PRIM. LENGTH	PRIMER SEQUENCE 5'- >3'	FRAG. LENGTH	SEQ ID NO:
	73R	ND2	5311	19	AGGGTGATGGTGGCTATGA	340	
20	74F	ND2	5234	15	CCCGCTAACCGGCTT	320	
	74R	ND2	5536	18	AGGGCTTTGAAGGCTCTT	320	
21	121F	TRNAs Trp/Ala	5449L	17	CCACACTCATCGCCCTT	287	
	121R	TRNAs Trp/Ala	5714H	22	GAGAAGTAGATTGAAGCCAGTT	287	
22	122F	tRNAs Asn/Cys/Tyr+O-L	5651L	20	CCCTTACTAGACCAATGGGA	322	
	122R	tRNAs Asn/Cys/Tyr+O-L	5950H	23	GCCGAATAATAGGTATAGTGTTC	322	
23	11F	CO I	5864	21	GTCCAATGCTTCACTCAGCCA		
	11FA	CO I	5859	21	TTACAGTCCAATGCTTCACTC	340	
	11R	CO I	6177	18	TATGCGGGGAAACGCCAT		
	11RA	CO I	6180	19	TGTTTATGCGGGGAAACGC	340	
24	12F	CO I	6138	21	GGCAACTGACTAGTTCCCCTA		
	12FA	CO I	6125	21	AATCGGAGGCTTTGGCAACTG	318	
	12R	CO I	6425	21	GTTTGGTATTGGGTTATGGCA		
	12RA	CO I	6422	21	TGGTATTGGGTTATGGCAGGG	318	
25	13F	CO I	6383	20	GGCCATCAATTTCATCACAA		
	13FA	CO I	6358	22	TAGCAGGTGTCTCCTCTATCTT	366	
	13R	CO I	6697	25	ATACCTATGTATCCAAATGGTTCTT		
	13RA	CO I	6699	25	CCATACCTATGTATCCAAATGGTTC	366	
26	14F	CO I	6657	25	GGAATAATCTCCCATATTGTAACTT	308	
	14R	CO I	6945	18	CAGGCCACCTACGGTGAA		
	14RA	CO I	6947	18	GTCAGGCCACCTACGGTG	308	
27	15F	CO I	6914	20	AGTGCTCTGAGCCCTAGGAT		
	15FA	CO I	6902	20	ATGATCTGCTGCAGTGCTCT	309	
	15R	CO I	7193	18	ATTCCGGATAGGCCGAGA	309	
28	16F	CO I	7159	22	TCGGCGTAAATCTAACTTTCTT	332	
	16R	CO I	7451	19	GGGGTTCGATTCCTTCCTT		
	16RA	CO I	7472	19	TTGGCTTGAAACCAGCTTT	332	
29	123F	TRNAs Ser/Asp	7373L	23	AAACCTGGAGTGACTATATGGAT	306	
	123R	TRNAs Ser/Asp	7659H	20	AATGATTATGAGGGCGTGAT	306	
30	21F	CO II	7546	24	TTGTCAAAGTTAAATTATAGGCTA		
	21FA	CO II	7548	24	GTCAAAGTTAAATTATAGGCTAAA	309	
	21R	CO II	7832	23	ACCTCGTCTGTTATGTAAAGGAT		
	21RA	CO II	7834	23	TGACCTCGTCTGTTATGTAAAGG	309	
31	22F	CO II	7792	20	CGCCATCATCCTAGTCCTCA	281	
	22R	CO II	8050	23	ATGAGTGCAAGACGTCTTGTGAT	281	
32	23F	CO II	8003	23	AATCGAGTAGTACTCCCGATTGA		
	23FA	CO II	8007	23	GAGTAGTACTCCCGATTGAAGCC	304	
	23R	CO II	8286	24	GTTAGCTTTACAGTGGGCTCTAGA		
	23RA	CO II	8287	24	AGTTAGCTTTACAGTGGGCTCTAG	304	
33	124F	tRNA Lys	8204L	19	ATGCCCATCGTCCTAGAAT	263	
	124R	tRNA Lys	8441H	26	TGGTAGTTTGTGTTTAATATTTTTAG	263	
34	41F	ATPase 8	8292	23	GCCCACTGTAAAGCTAACTTAGC	361	

FRAG	PRIMER	GENE	NUCLEOTIDE	PRIM. LENGTH	PRIMER SEQUENCE 5'- >3'	FRAG. LENGTH	SEQ ID NO:
	41R	ATPase 8	8631	22	TAGTCGGTTGTTGATGAGATAT	361	
	41RS	ATPase 8	8632	17	AGTCGGTTGTTGATGAG		
35	42F	ATPase 6	8572	23	GGCCTACCCGCCGCAGTACTGAT	360	
	42R	ATPase 6	8909	23	TGTAGGTGTGCCTTGTGGTAAGA	360	
36	43F	ATPase 6	8866	22	ATTATAGGCTTTCGCTCTAAGA		
	43FA	ATPase 6	8806	21	CCAACCACCCAACTATCTATA	430	
	43R	ATPase 6	9214	22	ATATGATAGGCATGTGATTGGT	430	
N/A	26F	ATPase 8	8311	25	TAGCATTAACCTTTTAAGTTAAAGA	224	
	26R	ATPase 8	8516	19	TCGTTCATTTTGGTTCTCA	224	
37	31F	CO III	9171	24	ACTTCTAGTAAGCCTCTACCTGCA		
	31FA	CO III	9173	24	TTCTAGTAAGCCTCTACCTGCACG	295	
	31R	CO III	9447	25	AGGTAATAAATAGGATTATCCCGTA		
	31RA	CO III	9443	25	AATAAATAGGATTATCCCGTATCGA	295	
38	32F	CO III	9416	19	CCACACACCACCTGTCCAA		
	32FA	CO III	9415	19	ACCACACACCACCTGTCCA	349	
	32R	CO III	9741	23	AAGGGAGACTCGAAGTACTCTGA	349	
39	33F	CO III	9712	23	TGGGTCTCTATTTTACCCTCCTA		
	33FA	CO III	9698	23	TATTACAATTTTACTGGGTCTCT	337	
	33R	CO III	10010	25	ACTAGTTAATTGGAAGTTAACGGTA	337	
40	125F	tRNA Glu	9932L	21	GCATTTTGTAGATGTGGTTTG	221	
	125R	tRNA Glu	10132H	21	CTATGTAGCCGTTGAGTTGTG	221	
41	75F	ND3	10007	25	TAGTACCGTTAACTTCCAATTAACT	374	
	75R	ND3	10430	24	TCATAATTTAATGAGTCGAAATCA	374	
42	126F	tRNA Arg	10341L	21	ATCATCATCCTAGCCCTAAGT	205	
	126R	tRNA Arg	10522H	24	GGTGTGAGCGATATACTAGTATTC	205	
43	81F	ND4L	10433	25	TTTCGACTCATTAAATTATGATAAT	365	
	81R	ND4L	10782	25	CATGTCAGTGGTAGTAATATAATTG	365	
44	82F	ND4	10718	22	CACATATGGCCTAGACTACGTA	368	
	82R	ND4	11060	23	ATAATTAAGGAGATTTGTAGGGA	368	
45	83F	ND4	10999	19	CCAACGCCACTTATCCAGT	364	
	83R	ND4	11342	25	AAGCTATTGTGTAAGCTAGTCATAT	364	
46	84F	ND4	11275	25	CTCACTAAACATTCTACTACTCACT	362	
	84R	ND4	11618	21	GTGGCTGATTGAAGAGTATGC	362	
47	85F	ND4	11554	24	CCTATGAGGCATAATTATAACAAG	336	
	85R	ND4	11894	22	ACGTGGTTACTAGCACAGAGAG	336	
48	86F	ND4	11834	19	TGACTTCTAGCAAGCCTCG	342	
	86R	ND4	12147	23	ACAATCTGATGTTTTGGTTAAAC	342	
49	127F	TRNAs His/Ser/Leu	12052L	19	ACGAGAAAACACCCTCATG	358	
	127R	TRNAs His/Ser/Leu	12391H	20	TTAACGAGGGTGGTAAGGAT	358	
50	93F	ND5	12281	21	CAGCTATCCATTGGTCTTAGG	415	
	93R	ND5	12671	25	TATTTGAAGAACTGATTAATGTTTG	415	
51	94F	ND5	12612	21	AGCATTGTTCGTTACATGGTC	353	
	94R	ND5	12944	21	GGCTTGGATTAGCGTTTAGAA	353	

FRAG	PRIMER	GENE	NUCLEOTIDE	PRIM. LENGTH	PRIMER SEQUENCE 5'- >3'	FRAG. LENGTH	SEQ ID NO:
52	95F	ND5	12881	20	TCATCCTCGCCTTAGCATGA	354	
	95R	ND5	13212	23	TTTTGATGTCATTTTGTGTAAGG	354	
53	96F	ND5	13156	23	CAAACTCTAACACTATGCTTAGG	383	
	96R	ND5	13519	20	ATGTTTGCGGTTTCGATGAT	383	
54	97F	ND5	13458	20	CATTGGCAGCCTAGCATTAG	423	
	97R	ND5	13855	26	GATTTTATTTTAAGTTTGTTGGTTAG	423	
55	98F	ND5	13795	19	AAACTCACAGCCCTCGCTG	353	
	98R	ND5	14124	24	TATGTGATTAGGAGTAGGGTTAGG	353	
56	91F	ND6	14119	22	CTCATCCTAACCCTACTCCTAA	329	
	91R	ND6	14442	19	GCGATGGCTATTGAGGAGT	329	
57	92F	ND6	14384	20	GCTAACCCCACTAAAACACT	447	
	92R	ND6	14693	20	TTCATATCATTGGTCGTGGT	447	
58	128F	TRNA Glu/Cyt B	14692L	20	AACCACGACCAATGATATGA	372	
	128R	TRNA Glu/Cyt B	15044H	20	GATCCGTAATATAGGCCTCG	372	
59	129F	Cyt B	14981L	17	ATCCGCTACCTTCACGC	376	
	129R	Cyt B	15339H	18	CCGTTTCGTGCAAGAATA	376	
60	130F	Cyt B	15279L	23	TCTTTACCTTTCACTTCATCTTG	374	
	130R	Cyt B	15632H	21	TGCTAGGATGAGGATGGATAG	374	
61	131F	Cyt B	15557L	21	GAATGATATTTCCTATTCGCC	342	
	131R	Cyt B	15910H	19	CCGGTTTACAAGACTGGTG	342	
62	132F	TRNA Thr/Phe	15841L	24	AATACCAACTATCTCCCTAATTGA	270	
	132R	TRNA Thr/Phe	16089H	22	CTGGCAGTAATGTACGAAATAC	270	
63	51F	D-LOOP	15968	22	TCTTTAACTCCACCATTAGCAC	442	
	51R	D-LOOP	16388	22	AGGATGGTGGTCAAGGGACCCC	442	

Sequencing of the purified PCR products was performed as described in Example 2, and 10-12 clones were sequenced for each cloned PCR product. Mutations were identified by comparison with published sequences including related corrections and reported polymorphisms for human mtDNA, as described above (Anderson et al., 1981 *Nature* 290:457; Andrews et al., 1999 *Nature Genetics* 23:147; Mitomap, www.gen.emory.edu). Identical, homoplasmic mtDNA mutations were detected in DNA sampled from D-1 white blood cells and in DNA from AD-1 cybrid cells. As shown in Table 2, polymorphisms, rare polymorphisms and sequence "errors" relative to the CRS (as described above) were detected. In addition, three novel, homoplasmic single nucleotide polymorphisms were detected as mtDNA mutations (Table 2): T1189C was observed in the 12S rRNA gene, G6366A was present in the CO1 (COX1) gene, and T12954C was found in the ND5 gene. Sequence analysis indicated that the G6366A mutation causes a valine→isoleucine substitution (missense) mutation at amino acid position 155 in the COX1 subunit of Complex IV, and that T12954C, by contrast, apparently represents a silent mutation in the ND5 subunit of Complex I.

EXAMPLE 5

EXPRESSION OF AD DONOR-DERIVED MITOCHONDRIAL DNA IN A CYBRID CELL LINE

This example shows expression of AD donor-derived mtDNA sequences in the AD-1 cybrid cell line constructed with mtDNA from a donor diagnosed as having AD, as described in Example 4.

A competitive primer extension assay was employed essentially as described (Fahy et al., 1997 *Nucl. Ac. Res.* 25:3102), but exploiting the homoplasmic G6366A transition mutation detected in AD-1 cybrid cells (described in Example 4). Template DNA was prepared from white blood cells and from cybrid cells as described in Example 4, and was also prepared from parental SH-SY5Y neuroblastoma cells and from pooled control cybrid cells, *i.e.*, cybrid cells constructed according to Miller et al. (1996 *J. Neurochem.* 67:1897-1907) but repopulated with mtDNA from subjects diagnosed as not having AD. To assess expression of the mtDNA-encoded COX1 (COI) gene, total cellular RNA was isolated from cybrid cells using Trizol™ reagent (Life Technologies, Inc., Gaithersburg, MD) and reverse transcribed with Superscript™ reverse transcriptase (Life Technologies) according to the manufacturer's instructions, to generate cDNA.

The following 5'-FAM labeled oligonucleotide primer was prepared according to standard methods:

5'--TGATGAAATTGATGGCCCCTAAGATAGAGGAGA--3' [SEQ ID NO:__]

A nucleotide mixture comprising dTTP, ddATP and ddCTP was used, such that wildtype (*i.e.*, CRS) mtDNA template directed extension of the primer with a ddC residue while D-1 (*i.e.*, AD-derived) mtDNA template directed extension of the primer with, sequentially, dT and ddA residues. As described in Fahy et al. (1997), primer extension reaction products were electrophoretically resolved under conditions permitting determination of differentially extended (*e.g.*, by one or by two nucleotides) fluorescent primer products. As shown in Figure 2, template DNA or cDNA from AD-1 cybrid cells (lanes 5 and 7) and from D-1 (AD) white blood cells (lane 4) directed the generation exclusively of primer extension products that were extended by two nucleotide residues, consistent with the presence of the homoplasmic G6366A mutation in mtDNA from these cells. By contrast, template DNA or cDNA from pooled control cybrid cells or from parental SH-SY5Y cells (lanes 6, 8 and 9) directed the generation only of primer extension products having a single nucleotide extension, indicative of the presence of wildtype DNA in these cells.

EXAMPLE 6

MITOCHONDRIAL ELECTRON TRANSPORT CHAIN ENZYMES IN AD-1 CYBRID CELLS

This example describes mitochondrial electron transport chain enzyme expression and activity in a cybrid cell model of Alzheimer's Disease.

AD-1 cybrid cells were prepared as described in Example 4. A control cybrid cell line was constructed by PEG fusion of platelets from three age-matched, cognitively normal subjects ρ^0 SH-SY5Y neuroblastoma cells, as described (Miller et al., 1996 *J. Neurochem.* 67:1897). The control cybrid cells had normal Complex I and IV activities relative to parental SH-SY5Y cells when assayed (see below) 32-37 days after fusion. Subsequently, an equal number of cells from each control cybrid cell line was pooled and this mixed control culture was maintained. Complex I (ND) and Complex IV (COX) activities were determined as described (Miller et al., 1996 *J. Neurochem.* 67:1897) and are reported as min-1mg-1 total cellular protein. Reactive oxygen species (ROS) were detected using the dichlorofluorescin diacetate (DCF-DA) assay (Miller et al., 1996). Activities of catalase, total superoxide dismutase (SOD), Mn SOD, Cu/Zn SOD, glutathione peroxidase and glutathione reductase were determined as previously described (Cassarino et al., 1997 *Biochim. Biophys. Acta* 1362:77). For determination of 8-hydroxyguanosine (8OH-dG) levels, DNA samples were prepared by hypo-osmotic lysis of cybrid cells, followed by proteolysis at 95°C with thermostable alkaline protease. The DNA was digested with exonuclease P1 and endonuclease III, and analyzed for 8OH-dG using electrochemical detection. Hydroxyl radical was assayed using thiobarbituric acid (Sattler et al., 1998 *Meths. Mol. Biol.* 110:167-191).

Complex I activity in AD-1 cybrid cells was essentially identical to that detected in mixed control cybrid cells. By contrast, Complex IV (COX) activity was markedly depressed in AD-1 cybrid cells relative to the mixed control cybrid cells, and this deficiency was stably maintained over extended culture periods (Figure 3). To determine whether the decreased Complex IV activity in AD-1 cybrids might be due to decreased amounts of COX protein in these cells (for example, reduced COX production related to the G6366A mutation in the COX1 subunit), expression levels of COX subunits 1, 2 and 4 were compared at the polypeptide level by western immunoblot analysis. Detergent lysates of AD-1 cybrid cells, mixed control cybrid cells and SH-SY5Y neuroblastoma cells were resolved electrophoretically, blot-transferred to nitrocellulose and probed with mouse anti-human COX subunit-specific antibodies (Molecular Probes, Inc., Eugene, OR) according to established procedures (Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing, 1987).

Blots were developed with HRP-conjugated anti-mouse immunoglobulin (Amersham, Inc., Arlington Heights, IL) and ECL chemiluminescent detection (Amersham) according to the supplier's instructions. As shown in Figure 4, AD-1 cybrid cells, mixed control cybrid cells and parental SH-SY5Y neuroblastoma cells all express comparable levels of the COX subunits. Without wishing to be bound by theory, these results suggest that a COX catalytic defect or defects in the ability of COX submits to associate with one another or to localize to the proper subcellular location (and not reduced levels of COX biosynthesis) may be a consequence of the G6366A mutation in the COX1 subunit of AD-1 cybrid cells.

AD-1 cybrid cells also exhibited elevated levels of reactive oxygen species (ROS) production, relative to the mixed control cybrid cells (Table 8), with an apparent gradual decline in the relative level of elevated ROS over time. To determine whether compensatory mechanisms might be involved in this gradual decline, AD-1 and mixed control cybrid cells were pre-loaded with 30 μM DCF-DA for 2 hours, rinsed, and then treated for 30 minutes with either 50 μM ethacrynic acid (a reduced glutathione scavenger) or with 2.5 mM aminotriazole (an inhibitor of the radical-savenging enzyme catalase) prior to ROS determination by the DCF-DA method. As shown in Figure 5, interfering with radical scavengers by treatment with either ethacrynic acid or aminotrialoze resulted in elevated ROS levels in AD-1 cells, relative to the ROS levels in mixed control cybrid cells. The oxidative buffering activity levels of multiple specific radical scavenger enzymes was also determined to be elevated in AD-1 cybrid cells relative to mixed control cybrids (Figure 6). Gene expression in the AD-1 cybrids relative to the control cybrids was investigated for two of these enzymes, Cu/Zn SOD and Mn SOD. As also shown in Fig. 6, the elevated activity levels for these enzymes were accompanied by elevated expression levels, suggesting a possible compensatory mechanism related to the apparent oxidative defects in the AD-1 cybrids.

Table 8

ROS PRODUCTION IN AD-1 CYBRID DECLINES OVER TIME

Days from Fusion	Fold Increase Over Pooled Cybrid Controls		
	ROS	8-Hydroxyguanosine	Thiobarbituric Acid
64	2.03		
87	1.42	8	8.5
113	1.44		
127	1.64		
143	0.96		
164	1.15		

EXAMPLE 7

HOMOPLASMIC SINGLE NUCLEOTIDE POLYMORPHISMS IN THE MITOCHONDRIAL DNA OF A SUBJECT DIAGNOSED WITH ALZHEIMER'S DISEASE

This example describes detection of mtDNA single nucleotide polymorphisms in DNA obtained from white blood cells of a second subject (D-2) diagnosed as having Alzheimer's Disease, and in a cybrid cell line prepared using platelet-derived mtDNA from the same subject. Materials and methods were identical to those described in Example 4, except that a different donor, D-2, was the source of white blood cells and platelets, and that a second AD cybrid cell line, AD-2, was constructed. The results are presented in Table 3. A novel, homoplasmic mtDNA mutation was identified in the 12S rRNA-encoding region of the mitochondrial genome, T980C.

EXAMPLE 8

SINGLE NUCLEOTIDE POLYMORPHISMS IN MITOCHONDRIAL RIBOSOMAL RNA GENES

In this example, analysis of mtDNA sequences for homoplasmic mutations in 12S and 16S rRNA genes is described. Brain and/or blood samples were obtained from 13 normal living control subjects, 41 autopsy-confirmed normal and neurological disease control (*i.e.*, non-AD) subjects, 13 living AD subjects, and 45 autopsy-confirmed AD and LBV (Lewy body variant) subjects.

DNA isolation from brain and blood samples was essentially as described in Examples 1 and 4, respectively, except that for some brain samples mitochondria were first purified and mtDNA extracted as described by Mecocci et al. (1994 *Ann. Neurol.* 36:747). Oligonucleotide primers specific for 12S rRNA and

16SrRNA genes are listed in Tables 5 and 7, and were used for PCR amplification, cloning and sequencing as described above.

Ten fragments generated by PCR amplification with primer sets 110-119 were amplified, cloned and sequenced as described in Examples 1 and 4. Homoplasmic single nucleotide polymorphisms detected in these mtDNA regions and the frequency of occurrence of these mutations are presented in Table 4, which includes mutations that are neither "polymorphisms" nor "rare polymorphisms" and that are also not "errors", as these terms are described above in the context of Tables 2-4. These include G709A, G930A, T980C, T1189C, T1243C, C1700T, G1719A, T1809C, A1811G, G1888A, G2098A, T2158C, C2259T, T2352C, G3010A, T3197C and a deletion of base 960, and also include T669C, T789C, C870T, T980C, G1007A, T1243C, G1393A, G1709A, G1719A, T2156C, A2294G, T2483C, A2581G, A2851G and an insertion of a T at base position 793.

Analysis of the data presented in Table 4 as a function of age indicated that, in the subject groups studied, differences in the load of mtDNA 12S/ 16S rRNA region homoplasmic single nucleotide polymorphisms were most pronounced when AD and control subjects 85 years old or older were compared (Figure 7). A trend toward a greater number of single nucleotide polymorphisms in mitochondrial rRNA genes in AD cases relative to control subjects was detectable in all age groups analyzed (64-74 years, 75-84 and 85 or older).

EXAMPLE 9

SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN MITOCHONDRIAL DNA THAT SEGREGATE WITH ALZHEIMER'S DISEASE

Mitochondrial DNA (mtDNA) was isolated and sequenced from brain and blood samples of 24 controls and 24 autopsy-confirmed AD cases. Confirmed AD samples consisted of 11 frontal cortex samples, 3 parietal cortex samples and 10 blood samples. Control samples consisted of 12 autopsy-confirmed brain samples (mean age 79.3 years: 5 normal controls, frontal cortex; 5 diffuse Lewy body dementia (DLBD) samples, frontal cortex; 2 parasupranuclear palsy (PSP) samples, frontal cortex) and 12 blood samples from healthy, normal volunteers (mean age 88.8 years) with no family history of AD.

Isolation of DNA by organic extraction following proteinase K/SDS solubilization was as described in Example 1. PCR amplification was performed as described in Example 2, except the oligonucleotide primer set presented in Table 10 was used to generate 68 PCR product fragments spanning the complete mtDNA

molecule, each fragment having approximately 50% sequence overlap with each neighboring product fragment. This strategy permitted direct mtDNA sequencing in both forward and reverse directions with four-fold redundancy in the identification of each nucleotide base, resulting in error-free sequencing. Thus, for each patient sample approximately 68,000 nucleotides were sequenced and analysis of homoplasmic mutations was verified.

Table 10

OLIGONUCLEOTIDE PRIMERS SPECIFIC FOR INDICATED REGIONS OF MITOCHONDRIAL GENOME

FRAGMENT	PRIMER	GENE	NUCLEOTIDE	PRIM. LENGTH	PRIMER SEQUENCE 5'->3'	SEQ ID NO.	FRAG. LENGTH
201	201F	D-Loop	16225L	24	CAACTATCACACATCAACTGCAAC		512
	201R		168H	21	TCGCCTGTAATATTGAACGTA		
202	202F	D-Loop	16477L	23	GCTAAAGTGAACTGTATCCGACA		379
	202R		287H	21	GACTGTTAAAAGTGCATACCG		
203	203F	D-Loop	155L	21	TATTTATCGCACCTACGTTCA		407
	203R		562H	18	AACTGTGGGGGGTGTCTT		
204	204F	D-Loop/tRNA Phe/12S	275L	23	GACATGATAACAAAAAATTTCCA		510
	204R		785H	18	GTGTGGCTAGGCTAAGCG		
205	205F	D-Loop/tRNA Phe/12S	498L	16	CGCCCATCCTACCCAG		541
	205R		1039H	25	TCTTAGCTATTGTGTGTTCAGATAT		
206	206F	12S	773L	19	TGCAGCTCAAAACGCTTAG		525
	206R		1298H	18	CGTGGGTACTTGCGCTTA		
207	207F	12S	1031L	24	GCTTTAACATATCTGAACACACAA		505

FRAGMENT	PRIMER	GENE	NUCLEOTIDE	PRIM. LENGTH	PRIMER SEQUENCE 5'->3'	SEQ ID NO.	FRAG. LENGTH
	207R		1536H	24	CTTGTCTCCTCTATATAAATGC GT		
208	208F	12S/tRNA Val/16S	1285L	20	GAAGGCTACAAAGTAAGCGC		500
	208R		1785H	17	TCATCTTTCCCTTGCGG		
209	209F	12S/tRNA Val/16S	1535L	24	TACGCATTTATATAGAGGAGA CAA		461
	209R		1996H	18	ATCACCAGGCTCGGTAGG		
210	210F	16S	1780L	19	TAGTACCGCAAGGGAAAGA		417
	210R		2197H	19	GTTGAGCTTGAACGCTTTC		
211	211F	16S	1986L	17	AGGCGACAAACCTACCG		411
	211R		2397H	23	GTGAGGGTAATAATGACTTGTT G		
212	212F	16S	2165L	21	CCATAGTAGGCCTAAAAGCAG		420
	212R		2585H	22	AGTGATTATGCTACCTTTGCAC		
213	213F	16S	2380L	24	CAATATCTACAARCAACCAAC AAG		413
	213R		2793H	20	ACCGAAATTTTTAATGCAGG		
214	214F	16S	2580L	19	TAACCGTGCAAAGGTAGCA		405
	214R		2985H	18	CCTGATCCAACATCGAGG		
215	215F	16S	2779L	21	CCTAAACTACCAAACCTGCAT		442

FRAGMENT	PRIMER	GENE	NUCLEOTIDE	PRIM. LENGTH	PRIMER SEQUENCE 5'->3'	SEQ ID NO.	FRAG. LENGTH
	215R		3221H	20	GCCATCTTAACAAACCTGT		
216	216F	16S/tRNA Leu/ND1	2974L	19	AGGGTTTACGACCTCGATG		517
	216R		3491H	17	GGTAGATGTGGCGGGTT		
217	217F	16S/tRNA Leu/ND1	3228L	19	TTGTTAAGATGGCAGAGCC		506
	217R		3734H	20	AATGATGGCTAGGGTGACTT		
218	218F	ND1	3482L	16	AGCCCCTAAAACCCGC		498
	218R		3980H	22	ATAATGTTTGTGTATTCGGCTA		
219	219F	ND1	3718L	23	CAAACAATCTCATATGAAGTC AC		520
	219R		4238H	17	AGGGGGAATGCTGGAGA		
220	220F	ND1/tRNAs Ile/Gln/Met/N D2	3967L	19	GCCCTATTCTTCATAGCCG		514
	220R		4481H	15	ATGACGGGTTGGGCC		
221	221F	ND1/tRNAs Ile/Gln/Met/N D2	4224L	21	CATACCCATTACAATCTCCAG		511
	221R		4735H	26	TATTAATGATGAGTATTGATTG GTAG		

FRAGMENT	PRIMER	GENE	NUCLEOTIDE	PRIM. LENGTH	PRIMER SEQUENCE 5'->3'	SEQ ID NO.	FRAG. LENGTH
222	222F	ND2	4481L	15	GGCCCAACCCGTCAT		508
	222R		4989H	20	GCTAAGATTTTGCGTAGCTG		
223	223F	ND2	4691L	23	CCTCTTCAACAATATACTCTCC G		548
	223R		5239H	16	GGCAAAAAGCCGGTTA		
224	224F	ND2	4979L	18	AAACCAGACCCAGCTACG		506
	224R		5485H	25	AAGATTATTAGTATAAAAGGG GAGA		
225	225F	ND2/tRNAs Trp/Ala/Asn	5234L	15	CCCGCTAACCGGCTT		480
	225R		5714H	22	GAGAAGTAGATTGAAGCCAGT T		
226	226F	ND2/tRNAs Trp/Ala/Asn/ Cys/Tyr+O-L	5455L	17	TCATCGCCCTTACCACG		537
	226R		5992H	19	AGGAGGCTTAGAGCTGTGC		
227	227F	tRNAs Ala/Asn/Cys/ Tyr+O-L/CO1	5700L	20	TAAGCACCCTAATCAACTGG		542
	227R		6242H	20	CCTCCACTATAGCAGATGCG		
228	228F	COI	5995L	21	CAGCTCTAAGCCTCCTTATTC		498

FRAGMENT	PRIMER	GENE	NUCLEOTIDE	PRIM. LENGTH	PRIMER SEQUENCE 5'->3'	SEQ ID NO.	FRAG. LENGTH
	228R		6493H	21	CAGCTAGGACTGGGAGAGATA		
229	229F	COI	6230L	19	CCTACTCCTGCTCGCATCT		527
	229R		6757H	21	TATGGTGTGCTCACACGATAA		
230	230F	COI	6476L	24	AGCAGTCCTACTTCTCCTATCT CT		510
	230R		6986H	25	GTCGTGTAGTACGATGTCTAGT GAT		
231	231F	COI	6713L	23	CATAGGTATGGTCTGAGCTATG A		517
	231R		7230H	18	GGTGTATGCATCGGGGTA		
232	232F	CO1/tRNA Ser	6979L	24	CAAACTCATCACTAGACATCGT AC		501
	232R		7480H	17	ATGGGGTTGGCTTGAAA		
233	233F	CO1/tRNAs Ser/Asp/COII	7225L	16	CGGACTACCCCGATGC		519
	233R		7744H	20	CCTGAGCGTCTGAGATGTTA		
234	234F	tRNAs Ser/Asp/COII	7469L	18	CCCAAAGCTGGTTTCAAG		510
	234R		7979H	18	GTCAAGGAGTCGCAGGTC		
235	235F	COII	7690L	20	CTTCCTAGTCCTGTATGCCC		557
	235R		8247H	18	GGGTAAATACGGGCCCTA		

FRAGMENT	PRIMER	GENE	NUCLEOTIDE	PRIM. LENGTH	PRIMER SEQUENCE 5'->3'	SEQ ID NO.	FRAG. LENGTH
236	236F	COII/tRNA Lys/ATPase 8	7975L	16	AGGCGACCTGCGACTC		504
	236R		8479H	21	TTTATTTTTATGGGCTTTGGT		
237	237F	COII/tRNA Lys/ATPase 8/ATPase 6	8225L	22	ATTCCCCTAAAAATCTTTGAAA		516
	237R		8741H	25	GCAATAAAAATGATTAAGGAT ACTA		
238	238F	ATPase 8/ATPase 6	8499L	24	AAAATTATAACAAACCCTGAG AAC		497
	238R		8996H	16	GCGGTTAGGCGTACGG		
239	239F	ATPase 8/ATPase 6/COIII	8722L	26	CGAACCTGATCTCTTATACTAG TATC		515
	239R		9237H	18	TCATGGGCTGGGTTTTAC		
240	240F	ATPase 6/COIII	8978L	18	TTCAACCAATAGCCCTGG		506
	240R		9484H	19	CAGAAAAATCCTGCGAAGA		
241	241F	COIII	9229L	22	ATCATATAGTAAAACCCAGCC C		505
	241R		9734H	21	TCGAAGTACTCTGAGGCTTGT		

FRAGMENT	PRIMER	GENE	NUCLEOTIDE	PRIM. LENGTH	PRIMER SEQUENCE 5'->3'	SEQ ID NO.	FRAG. LENGTH
242	242F	COIII/tRNA Glu	9470L	21	CTCAGAAGTTTTTTTCTTCGC		537
	242R		10007H	25	AGTTAATTGGAAGTTAACGGT ACTA		
243	243F	COIII/tRNA Glu/ND3	9732L	22	CTACAAGCCTCAGAGTACTTCG		520
	243R		10252H	21	GGAGGGCAATTTCTAGATCAA		
244	244F	COIII/tRNA Glu/ND3/tRN A Arg	9976L	24	ATTGATGAGGGTCTTACTCTTT TA		523
	244R		10499H	23	CTAGAAGTGAGATGGTAAATG CT		
245	245F	ND3/tRNA Arg/ND4L	10209L	26	TTCTCCATAAAATTCTTCTTAG TAGC		522
	245R		10731H	26	AGTAGGTTTAGGTTATGTACGT AGTC		
246	246F	tRNA Arg/ND4L/ND 4	10450L	24	ATGATAATCATATTTACCAAAT GC		536
	246R		10986H	18	GTTGGCTTGCCATGATTG		
247	247F	ND4L/ND4	10719L	23	ACATATGGCCTAGACTACGTAC A		511

FRAGMENT	PRIMER	GENE	NUCLEOTIDE	PRIM. LENGTH	PRIMER SEQUENCE 5'->3'	SEQ ID NO.	FRAG. LENGTH
	247R		11230H	18	GCGATGAGTAGGGGAAGG		
248	248F	ND4	10979L	17	CCCCTCACAATCATGGC		502
	248R		11481H	22	AGTGTGAGGCGTATTATACCAT		
249	249F	ND4	11213L	20	TACACCCTAGTAGGCTCCCT		524
	249R		11737H	21	TTTGAGTTTGCTAGGCAGAAT		
250	250F	ND4	11474L	20	GGCGGCTATGGTATAATACG		510
	250R		11984H	24	CCATTGTGTTGTGGTAAATATGTA		
251	251F	ND4/tRNAs His/Ser	11721L	24	TTACATCCTCATTACTATTCTGCC		511
	251R		12232H	18	TTAGACATGGGGGCATGA		
252	252F	ND4/tRNAs His/Ser/Leu/ ND5	11968L	23	AGCCCTATACTCCCTCTACATAT		538
	252R		12506H	25	TTCGAGATAATAACTTCTTGGTCTA		
253	253F	tRNAs Ser/Leu/ND5	12213L	22	GCTCACAAGAACTGCTAACTCA		516
	253R		12729H	22	GAATAGGTTGTTAGCGGTAACT		
254	254F	ND5	12458L	24	CATCCACCTTTATTATCAGTCTCT		532

FRAGMENT	PRIMER	GENE	NUCLEOTIDE	PRIM. LENGTH	PRIMER SEQUENCE 5'->3'	SEQ ID NO.	FRAG. LENGTH
	254R		12990H	17	ATTTGCCTGCTGCTGCT		
255	255F	ND5	12714L	25	TACCATACTAATCTTAGTTACC GCT		520
	255R		13234H	21	AGTGGAGAAGGCTACGATTTT		
256	256F	ND5	12979L	17	GGCCTCCTCCTAGCAGC		501
	256R		13480H	21	ACCTGTGAGGAAAGGTATTCC		
257	257F	ND5	13225L	21	GACATCAAAAAAATCGTAGCC		504
	257R		13729H	25	AAATGTTGTTAGTAATGAGAA ATCC		
258	258F	ND5	13472L	21	CATTAGCAGGAATACCTTTCC		506
	258R		13978H	19	CTAGGAGGAGTAGGGGCAG		
259	259F	ND5/ND6	13720L	21	CTATTCGCAGGATTTCTCATT		517
	259R		14237H	16	GTGCGGGGGCTTTGTA		
260	260F	ND5/ND6	13939L	19	CGCACAATCCCCTATCTAG		545
	260R		14484H	22	TTAATTTATTTAGGGGGAATGA		
261	261F	ND6/tRNA Glu	14209L	22	AACTACTACTAATCAACGCCCA		522
	261R		14731H	22	GGTCATTGGTGTTCTTGTAGTT		
262	262F	ND6/tRNA Glu/Cyt b	14471L	20	CCAAAGACAACCATCATTCC		508

FRAGMENT	PRIMER	GENE	NUCLEOTIDE	PRIM. LENGTH	PRIMER SEQUENCE 5'->3'	SEQ ID NO.	FRAG. LENGTH
	262R		14979H	18	CGTGAAGGTAGCGGATGA		
263	263F	tRNA Glu/Cyt b	14715L	23	ACCATCGTTGTATTTCAACTAC A		514
	263R		15229H	21	TAGCCTCCTCAGATTCATTGA		
264	264F	Cyt b	14961L	22	ACGTAAATTATGGCTGAATCAT		518
	264R		15479H	20	CCTAGGAGGTCTGGTGAGAA		
265	265F	Cyt b	15223L	22	CCTAGTTCAATGAATCTGAGGA		509
	265R		15732H	17	AATGAGGAGGTCTGCGG		
266	266F	Cyt b/tRNA Thr/Phe	15449L	24	TTCCTTCTCTCCTTAATGACATT A		530
	266R		15979H	20	TAGCTTTGGGTGCTAATGGT		
267	267F	Cyt b/tRNAs Thr/Phe/D-Loop	15723L	18	GACTCCTAGCCGCAGACC		509
	267R		16232H	20	GGAGTTGCAGTTGATGTGTG		
268	268F	tRNA Phe/D-Loop	15968L	22	TCTTTAACTCCACCATTAGCAC		517
	268R		16485H	24	GGAACCAGATGTCGGATACAG TTC		

Sequencing was conducted using a Perkin-Elmer Model 3700 DNA Analyzer with 96-capillary array according to the manufacturer's instructions, and data analysis, performed essentially as described in Example 2, also included categorization of sample sequences according to various parameters, including: source of tissue sample, patient clinical status (*e.g.*, AD or control), patient haplogroup, mtDNA gene region in which an identified SNP resided and, for protein encoding mtDNA genes in which an AD-associated SNP was identified, whether the SNP was a synonymous substitution (*i.e.*, resulted in no change in the amino acid sequence of the encoded protein) or a non-synonymous substitution (*i.e.*, resulted in a different amino acid sequence for the encoded protein).

AD-associated SNPs are presented in Table 9. Figures 8-14 depict the results of quantitative data analyses wherein AD-associated SNPs were grouped according to selected parameters. Thus, Fig. 8 presents a profile of the samples analyzed in this Example according to mtDNA haplogroup. In Fig. 9, the distribution (percentage of samples analyzed having one or more SNP per indicated gene region) of AD-associated SNPs from Table 9 that reflect non-synonymous nucleotide substitutions according to mtDNA protein coding regions (*i.e.*, gene loci) is presented, wherein SNPs detected in the AD group are compared to SNPs detected in all non-AD control groups.

Table 9

MTDNA SINGLE NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH AD

Gene	Nucleotide Position	Nucleotide (crs)	Nucl. Substitution	# Samples	Haplogroup
D-LOOP	72	T	C	1	H
D-LOOP	114	C	T	1	K
D-LOOP	146	T	C	2	U, H
D-LOOP	185	G	A	1	J
D-LOOP	189	A	G	1	K, W
D-LOOP	199	T	C	1	I
D-LOOP	204	T	C	1	W
D-LOOP	207	G	A	2	W, I
D-LOOP	228	G	A	1	J
D-LOOP	236	T	C	1	H
D-LOOP	239	T	C	2	H
D-LOOP	456	C	T	1	H
D-LOOP	462	C	T	2	J
D-LOOP	482	T	C	1	J
D-LOOP	489	T	C	2	J
D-LOOP	497	C	T	1	K, K
D-LOOP	500	C	G	5	H, W, J
D-LOOP	516	C	T	1	U
D-LOOP	522	C	DEL	1	H
D-LOOP	523	A	DEL	1	H
D-LOOP	547	A	T	1	I
12S RRNA	593	T	C	1	K
12S RRNA	669	T	C	1	I
12S RRNA	960	C	DEL	1	U
12S RRNA	1007	G	A	1	J
12S RRNA	1243	T	C	1	W
12S RRNA	1393	G	A	1	H
16S RRNA	1719	G	A	1	H, I
16S RRNA	1809	T	C	1	U
16S RRNA	2352	T	C	1	H
16S RRNA	2483	T	C	1	K
16S RRNA	2702	G	A	1	I

Gene	Nucleotide Position	Nucleotide (crs)	Nucl. Substitution	# Samples	Haplogroup
16S RRNA	2851	A	G	1	H
16S RRNA	3197	T	C	1	U
ND1	3333	C	T	1	H
ND1	3336	T	C	1	I
ND1	3348	A	G	1	U
ND1	3394	T	C	1	J
ND1	3398	T	C	1	I
ND1	3423	G	T	1	J
ND1	3505	A	G	1	W
ND1	3559	C	T	1	H
ND1	3915	G	A	2	H
ND1	3992	C	T	1	H
ND1	4024	A	G	1	H
ND1	4095	C	T	1	H
ND1	4216	T	C	3	T, J
TRNA-Q	4336	T	C	1	H
ND2	4529	A	T	1	I
ND2	4727	A	G	2	H
ND2	4793	A	G	1	H
ND2	4917	A	G	1	T
ND2	4991	G	A	1	H
ND2	5004	T	C	2	H, W
ND2	5046	G	A	1	W
ND2	5228	C	G	1	H
ND2	5315	A	G	1	I
ND2	5418	T	G	1	J
ND2	5426	T	C	1	T
ND2	5460	G	A	3	H, W
ND2	5461	C	G	1	J
TRNA-W	5516	A	G	1	H
TRNA-W	5554	C	A	1	U
TRNA-A	5634	A	G	1	H
TRNA-A/ TRNA-N	5656	A	G	1	U
TRNA-C	5773	G	A	1	J

Gene	Nucleotide Position	Nucleotide (crs)	Nucl. Substitution	# Samples	Haplogroup
CO1	6182	G	A	1	U
CO1	6221	T	C	1	H
CO1	6341	C	T	1	U
CO1	6367	T	C	1	K
CO1	6371	C	T	1	H
CO1	6489	C	A	1	T
CO1	7184	A	G	1	J
CO1	7325	A	G	1	H
CO2	7621	T	C	1	K
CO2	7768	A	G	1	U
CO2	7787	C	T	1	H
CO2	7789	G	A	1	J
CO2	7864	C	T	1	W
CO2	7895	G	A	1	U
CO2	7963	A	G	1	J
CO2	8149	A	G	1	H
CO2	8251	G	A	2	W, I
CO2	8269	G	A	1	H
CO2/ TRNA-K	8276-8284		DEL	1	T
ATPAse 8	8470	A	G	1	H
ATPASE 8	8485	G	A	1	I
ATPASE 8	8508	A	G	1	I
ATPASE 6	8602	T	C	1	H
ATPASE6	8697	G	A	1	T
ATPASE 6	8752	A	G	1	H
ATPASE 6	8901	A	G	1	I
ATPASE 6	8994	G	A	1	W
ATPASE 6	9123	G	A	1	H
CO3	9254	A	G	1	H
CO3	9362	A	G	1	H
CO3	9380	G	A	2	H
CO3	9477	G	A	1	U
CO3	9554	G	A	1	H
CO3	9708	T	C	1	H

Gene	Nucleotide Position	Nucleotide (crs)	Nucl. Substitution	# Samples	Haplogroup
CO3	9804	G	A	1	H
CO3	9861	T	C	1	H
TRNA-G	10034	T	C	1	I
TRNA-G	10044	A	G	1	H
ND3	10238	T	C	2	I
TRNA-R	10463	T	C	2	T, J
ND4L	10589	G	A	1	H
ND4	10978	A	G	1	K
ND4	11065	A	G	1	I
ND4	11251	A	G	1	J
ND4	11253	T	C	1	H
ND4	11272	A	G	1	U
ND4	11470	A	G	2	K
ND4	11527	C	T	1	J
ND4	11611	G	A	1	H
ND4	11674	C	T	1	W
ND4	11812	A	G	1	T
ND4	11914	G	A	2	K
ND4	11947	A	G	1	W
ND5	12414	T	C	1	W
ND5	12501	G	A	2	I
ND5	12609	T	C	1	U
ND5	12705	C	T	4	H, W, I
ND5	12954	T	C	1	K
ND5	13111	T	C	1	H
ND5	13194	G	A	2	H, U
ND5	13212	C	T	1	H
ND5	13368	G	A	1	T
ND5	13617	T	C	1	U
ND5	13780	A	G	2	I
ND5	13966	A	G	1	H
ND5	14020	T	C	1	T
ND5	14148	A	G	1	W
ND6	14178	T	C	1	I

Gene	Nucleotide Position	Nucleotide (crs)	Nucl. Substitution	# Samples	Haplogroup
ND6	14179	A	G	1	U
ND6	14182	T	C	1	U
ND6	14212	T	C	1	H
ND6	14233	A	G	1	T
ND6	14470	T	C	1	H
ND6	14582	A	G	1	H
CYT.B	14905	G	A	1	T
CYT.B	15028	C	A	1	T
CYT.B	15043	G	A	3	T, I
CYT.B	15191	T	C	1	U
CYT.B	15299	T	C	1	I
CYT.B	15380	A	G	1	U
CYT.B	15553	G	A	1	H
CYT.B	15607	A	G	1	T
CYT.B	15758	A	G	1	I
CYT.B	15790	C	T	1	U
CYT.B	15808	A	G	1	H
CYT.B	15833	C	T	1	H
CYT.B	15884	G	C	1	W
TRNA-T	15924	A	G	3	K, I
TRNA-T	15928	G	A	1	T
D-LOOP	16069	C	T	2	J
D-LOOP	16086	T	C	1	I
D-LOOP	16093	T	C	1	K
D-LOOP	16126	T	C	3	T, J
D-LOOP	16129	G	A	2	H, I
D-LOOP	16145	G	A	1	I
D-LOOP	16147	C	A	1	I
D-LOOP	16172	T	C	1	U
D-LOOP	16174	C	T	1	U
D-LOOP	16182	A	C	1	T
D-LOOP	16183	A	C	4	T, U, H
D-LOOP	16189	T	C	5	T, U, H
D-LOOP	16192	C	T	1	U

Gene	Nucleotide Position	Nucleotide (crs)	Nucl. Substitution	# Samples	Haplogroup
D-LOOP	16193	C	T	1	J
D-LOOP	16223	C	T	4	H, W, I
D-LOOP	16224	T	C	3	K
D-LOOP	16234	C	T	1	K
D-LOOP	16235	A	G	1	J
D-LOOP	16239	C	T	1	H
D-LOOP	16248	C	T	1	J
D-LOOP	16256	C	T	1	J
D-LOOP	16261	C	T	1	H
D-LOOP	16270	C	T	1	U
D-LOOP	16278	C	T	2	U, H
D-LOOP	16290	C	T	1	H
D-LOOP	16292	C	T	1	W
D-LOOP	16293	A	G	1	H
D-LOOP	16294	C	T	1	T
D-LOOP	16298	T	C	2	T, H
D-LOOP	16300	A	G	1	J
D-LOOP	16304	T	C	1	H
D-LOOP	16309	A	G	1	J
D-LOOP	16311	T	C	5	H, K, U
D-LOOP	16320	C	T	1	I
D-LOOP	16355	C	T	1	I
D-LOOP	16362	T	C	2	H
D-LOOP	16391	G	A	1	I
D-LOOP	16482	A	G	2	H
D-LOOP	16524	A	G	1	K

In Fig. 10, the distribution (percentage of samples analyzed having one or more SNP per indicated gene region) of AD-associated SNPs from Table 9 that reflect non-synonymous nucleotide substitutions according to mtDNA protein coding regions (*i.e.*, gene loci) is presented, wherein SNPs detected in the AD group are compared to SNPs detected in non-AD control groups that have been further categorized as normal (*i.e.*, healthy) or diseased controls.

Fig. 11 shows the distribution (percentage of samples analyzed having one or more SNP per indicated gene region) of AD-associated SNPs from Table 9 that reflect synonymous nucleotide substitutions according to mtDNA protein coding regions (*i.e.*, gene loci), wherein SNPs detected in the AD group are compared to SNPs detected in non-AD control groups that have been further categorized as normal (*i.e.*, healthy) or diseased controls.

Fig. 12 shows the distribution (percentage of samples analyzed having one or more SNP per indicated gene region) of AD-associated SNPs from Table 9 that reflect nucleotide substitutions in mitochondrial tRNA genes according to each of the 22 known human mitochondrial tRNA coding regions (*i.e.*, gene loci), wherein SNPs detected in the AD group are compared to SNPs detected in non-AD control groups that have been further categorized as normal (*i.e.*, healthy) or diseased controls.

Figure 13 summarizes the distribution (percentage of samples analyzed having one or more SNP per indicated mtDNA region) of AD-associated SNPs from Table 9 that reflect nucleotide substitutions in the indicated mtDNA region, wherein SNPs detected in the AD group are compared to SNPs detected in the corresponding mtDNA regions in samples derived from normal, healthy controls.

Figure 14 summarizes the distribution (number of SNPs detected per patient) of AD-associated SNPs from Table 9 that reflect nucleotide substitutions in the indicated mtDNA region, wherein SNPs detected in the AD group are compared to SNPs detected in the corresponding mtDNA regions in samples derived from normal, healthy controls.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

1. A method for determining the risk for or presence of Alzheimer's disease in a first subject suspected of having or being at risk for having such a disease, comprising:

determining the presence or absence of at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease in each of a first and a second biological sample comprising mitochondrial DNA, said first biological sample being obtained from said first subject and said second sample being obtained from a second subject known to be free of a risk or presence of a disease associated with altered mitochondrial function,

wherein the presence of at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease in said first biological sample and the absence of a mitochondrial single nucleotide polymorphism at a corresponding nucleotide in said second biological sample indicates an increased risk of Alzheimer's disease,

and therefrom determining the risk or presence of Alzheimer's disease.

2. The method of claim 1 wherein the mitochondrial DNA in the first sample is amplified and the mitochondrial DNA in the second sample is amplified.

3. The method of claim 1 wherein the step of determining comprises:

contacting each of said first and second biological samples with an oligonucleotide primer having a nucleotide sequence that is complementary to a sequence present in the mitochondrial DNA of said first sample and present in the mitochondrial DNA of said second sample, under conditions and for a time sufficient to allow hybridization of said primer to the mitochondrial DNA; and

detecting hybridization and extension of the primer to the mitochondrial DNA of the first sample to produce a first product and hybridization and extension of the primer to the mitochondrial DNA of the second sample to produce a second product distinguishable from said first product, and therefrom determining the presence or absence of at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease.

4. The method of claim 3 wherein the mitochondrial DNA in the first sample is amplified and the mitochondrial DNA in the second sample is amplified.

5. The method of claim 3 wherein at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease and present in said first biological sample and that is absent at a corresponding nucleotide in said second biological sample is present in a mitochondrial DNA region selected from the group consisting of a D-loop, a mitochondrial rRNA gene, a mitochondrial NADH dehydrogenase gene, a mitochondrial tRNA gene, a mitochondrial cytochrome c oxidase gene, a mitochondrial ATP synthase gene and a mitochondrial cytochrome b gene.

6. The method of claim 1 wherein at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease and present in said first biological sample and that is absent at a corresponding nucleotide in said second biological sample is present in a mitochondrial DNA region selected from the group consisting of a D-loop, a mitochondrial rRNA gene, a mitochondrial NADH dehydrogenase gene, a mitochondrial tRNA gene, a mitochondrial cytochrome c oxidase gene, a mitochondrial ATP synthase gene and a mitochondrial cytochrome b gene.

7. The method of claim 6 wherein at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease and present in said first biological sample and that is absent at a corresponding nucleotide in said second biological sample is present in a mitochondrial DNA region selected from the group consisting of a mitochondrial NADH dehydrogenase gene, a mitochondrial cytochrome c oxidase gene, a mitochondrial ATP synthase gene and a mitochondrial cytochrome b gene, and wherein the single nucleotide polymorphism is a non-synonymous nucleotide substitution.

8. The method of claim 6 wherein at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease and is present in said first biological sample and that is absent at a corresponding nucleotide in said second biological sample is present in a mitochondrial DNA region selected from the group consisting of a mitochondrial NADH dehydrogenase gene, a mitochondrial cytochrome c oxidase gene, a mitochondrial ATP synthase gene and a mitochondrial cytochrome b gene, and wherein the single nucleotide polymorphism is a synonymous nucleotide substitution.

9. The method of claim 6 wherein at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease and is present in said first biological sample and that is absent at a corresponding nucleotide in said second biological sample is a mitochondrial single nucleotide polymorphism located at a nucleotide

that corresponds to a nucleotide position of SEQ ID NO:1 that is selected from the group consisting of position 72, 114, 146, 185, 189, 199, 204, 207, 228, 236, 239, 456, 462, 482, 489, 497, 500, 516, 522, 523, 547, 593, 669, 960, 1007, 1243, 1393, 1719, 1809, 2352, 2483, 2702, 2851, 3197, 3333, 3336, 3348, 3394, 3398, 3423, 3505, 3559, 3915, 3992, 4024, 4095, 4216, 4336, 4529, 4727, 4793, 4917, 4991, 5004, 5046, 5228, 5315, 5418, 5426, 5460, 5461, 5516, 5554, 5634, 5656, 5773, 6182, 6221, 6341, 6367, 6371, 6489, 7184, 7325, 7621, 7768, 7787, 7789, 7864, 7895, 7963, 8149, 8251, 8269, 8276-8284, 8470, 8485, 8508, 8602, 8697, 8752, 8901, 8994, 9123, 9254, 9362, 9380, 9477, 9554, 9708, 9804, 9861, 10034, 10044, 10238, 10463, 10589, 10978, 11065, 11251, 11253, 11272, 11470, 11527, 11611, 11674, 11812, 11914, 11947, 12414, 12501, 12609, 12705, 12954, 13111, 13194, 13212, 13368, 13617, 13780, 13966, 14020, 14148, 14178, 14179, 14182, 14212, 14233, 14470, 14582, 14905, 15028, 15043, 15191, 15299, 15380, 15553, 15607, 15758, 15790, 15808, 15833, 15884, 15924, 15928, 16069, 16086, 16093, 16126, 16129, 16145, 16147, 16172, 16174, 16182, 16183, 16189, 16192, 16193, 16223, 16224, 16234, 16235, 16239, 16248, 16256, 16261, 16270, 16278, 16290, 16292, 16293, 16294, 16298, 16300, 16304, 16309, 16311, 16320, 16355, 16362, 16391, 16482 and 16524.

10. The method of claim 6 wherein at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease and is present in said first biological sample and that is absent at a corresponding nucleotide in said second biological sample is a mitochondrial single nucleotide polymorphism located at a nucleotide that corresponds to a nucleotide position of SEQ ID NO:1 that is selected from the group consisting of position 709, 930, 960, 980, 1189, 1243, 1700, 1719, 1809, 1811, 1888, 2098, 2158, 2259, 2352, 3010, 3197, 669, 789, 793, 870, 980, 1007, 1243, 1393, 1709, 1719, 2156, 2294, 2483, 2581, 2851, 6366 and 12954.

11. A method for determining the risk for or presence of Alzheimer's disease in a subject, comprising:

determining the presence of at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease in a biological sample comprising mitochondrial DNA from the subject.

12. The method of claim 11 wherein at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease is present in a mitochondrial DNA region selected from the group consisting of a D-loop, a mitochondrial rRNA gene, a mitochondrial NADH dehydrogenase gene, a mitochondrial tRNA gene, a

mitochondrial cytochrome c oxidase gene, a mitochondrial ATP synthase gene and a mitochondrial cytochrome b gene.

13. The method of claim 11 wherein at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease is present in a mitochondrial DNA region selected from the group consisting of a mitochondrial NADH dehydrogenase gene, a mitochondrial cytochrome c oxidase gene, a mitochondrial ATP synthase gene and a mitochondrial cytochrome b gene, and wherein the single nucleotide polymorphism is a non-synonymous nucleotide substitution.

14. The method of claim 11 wherein at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease is present in a mitochondrial DNA region selected from the group consisting of a mitochondrial NADH dehydrogenase gene, a mitochondrial cytochrome c oxidase gene, a mitochondrial ATP synthase gene and a mitochondrial cytochrome b gene, and wherein the single nucleotide polymorphism is a synonymous nucleotide substitution.

15. The method of claim 11 wherein at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease is a mitochondrial single nucleotide polymorphism located at a nucleotide that corresponds to a nucleotide position of SEQ ID NO:1 that is selected from the group consisting of position 72, 114, 146, 185, 189, 199, 204, 207, 228, 236, 239, 456, 462, 482. 489, 497, 500, 516, 522, 523, 547, 593, 669, 960, 1007, 1243, 1393, 1719, 1809, 2352, 2483, 2702, 2851, 3197, 3333, 3336, 3348, 3394, 3398, 3423, 3505, 3559, 3915, 3992, 4024, 4095, 4216, 4336, 4529, 4727, 4793, 4917, 4991, 5004, 5046, 5228, 5315, 5418, 5426, 5460, 5461, 5516, 5554, 5634, 5656, 5773, 6182, 6221, 6341, 6367, 6371, 6489, 7184, 7325, 7621, 7768, 7787, 7789, 7864, 7895, 7963, 8149, 8251, 8269, 8276-8284, 8470, 8485, 8508, 8602, 8697, 8752, 8901, 8994, 9123, 9254, 9362, 9380, 9477, 9554, 9708, 9804, 9861, 10034, 10044, 10238, 10463, 10589, 10978, 11065, 11251, 11253, 11272, 11470, 11527, 11611, 11674, 11812, 11914, 11947, 12414, 12501, 12609, 12705, 12954, 13111, 13194, 13212, 13368, 13617, 13780, 13966, 14020, 14148, 14178, 14179, 14182, 14212, 14233, 14470, 14582, 14905, 15028, 15043, 15191, 15299, 15380, 15553, 15607, 15758, 15790, 15808, 15833, 15884, 15924, 15928, 16069, 16086, 16093, 16126, 16129, 16145, 16147, 16172, 16174, 16182, 16183, 16189, 16192, 16193, 16223, 16224, 16234, 16235, 16239, 16248, 16256, 16261, 16270, 16278, 16290, 16292, 16293, 16294, 16298, 16300, 16304, 16309, 16311, 16320, 16355, 16362, 16391, 16482 and 16524.

16. The method of claim 11 wherein at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease is a mitochondrial single nucleotide polymorphism located at a nucleotide that corresponds to a nucleotide position of SEQ ID NO:1 that is selected from the group consisting of position 709, 930, 960, 980, 1189, 1243, 1700, 1719, 1809, 1811, 1888, 2098, 2158, 2259, 2352, 3010, 3197, 669, 789, 793, 870, 980, 1007, 1243, 1393, 1709, 1719, 2156, 2294, 2483, 2581, 2851, 6366 and 12954.

17. A method of determining if an agent is likely to cause, contribute to the pathology of, or exacerbate Alzheimer's disease, comprising contacting a biological sample comprising a cell with a candidate agent, conducting an assay of a mitochondrial nucleic acid to determine if said mitochondrial nucleic acid contains one or more single nucleotide polymorphisms after being contacted with said agent, wherein said nucleic acid is present in or derived from said cell and said one or more single nucleotide polymorphisms segregate with Alzheimer's disease.

18. A method of determining if an agent is likely to cause, contribute to the pathology of, or exacerbate Alzheimer's disease, comprising the steps of:

(a) contacting a first cell with a candidate agent;

(b) incubating a second cell, that has not been contacted with said agent, and said first cell, under equivalent conditions;

(c) conducting an assay of one or more mitochondrial nucleic acids to determine if said mitochondrial nucleic acids contain one or more single nucleotide polymorphisms, wherein said nucleic acids are present in or derived from said cells and said single nucleotide polymorphisms segregate with Alzheimer's disease,

wherein the presence of one or more of said single nucleotide polymorphisms in said mitochondrial nucleic acids present in or derived from said first cell, and the absence of one or more of said single nucleotide polymorphisms in said mitochondrial nucleic acids present in or derived from said second cell, indicates that said agent is likely to cause, contribute to the pathology of, or exacerbate Alzheimer's disease.

19. A nucleic acid array comprising a plurality of isolated nucleic acid molecules immobilized on a solid support, wherein said isolated nucleic acid molecules comprise all or a portion of the nucleic acid sequence set forth in SEQ ID NO:1 in which at

least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease is present.

20. The nucleic acid array of claim 19 wherein the mitochondrial single nucleotide polymorphism is located at a nucleotide that corresponds to a nucleotide position of SEQ ID NO:1 that is selected from the group consisting of position 72, 114, 146, 185, 189, 199, 204, 207, 228, 236, 239, 456, 462, 482. 489, 497, 500, 516, 522, 523, 547, 593, 669, 960, 1007, 1243, 1393, 1719, 1809, 2352, 2483, 2702, 2851, 3197, 3333, 3336, 3348, 3394, 3398, 3423, 3505, 3559, 3915, 3992, 4024, 4095, 4216, 4336, 4529, 4727, 4793, 4917, 4991, 5004, 5046. 5228, 5315, 5418, 5426, 5460, 5461, 5516, 5554, 5634, 5656, 5773, 6182, 6221, 6341, 6367, 6371, 6489, 7184, 7325, 7621, 7768, 7787, 7789, 7864, 7895, 7963, 8149, 8251, 8269, 8276-8284, 8470, 8485, 8508, 8602, 8697, 8752, 8901, 8994, 9123, 9254, 9362, 9380, 9477, 9554, 9708, 9804, 9861, 10034, 10044, 10238, 10463, 10589, 10978, 11065, 11251, 11253, 11272, 11470, 11527, 11611, 11674, 11812, 11914, 11947, 12414, 12501, 12609, 12705, 12954, 13111, 13194, 13212, 13368, 13617, 13780, 13966, 14020, 14148, 14178, 14179, 14182, 14212, 14233, 14470, 14582, 14905, 15028, 15043, 15191, 15299, 15380, 15553, 15607, 15758, 15790, 15808, 15833, 15884, 15924, 15928, 16069, 16086, 16093, 16126, 16129, 16145, 16147, 16172, 16174, 16182, 16183, 16189, 16192, 16193, 16223, 16224, 16234, 16235, 16239, 16248, 16256, 16261, 16270, 16278, 16290, 16292, 16293, 16294, 16298, 16300, 16304, 16309, 16311, 16320, 16355, 16362, 16391, 16482, 16524, 709, 930, 960, 980, 1189, 1243, 1700, 1719, 1809, 1811, 1888, 2098, 2158, 2259, 2352, 3010, 3197, 669, 789, 793, 870, 980, 1007, 1243, 1393, 1709, 1719, 2156, 2294, 2483, 2581, 2851, 6366 and 12954.

21. The method of claim 1 wherein the step of determining comprises:

contacting each of said first and second biological samples with an oligonucleotide primer comprising all or a portion of the nucleic acid sequence set forth in SEQ ID NO:1 in which at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease is present, under conditions and for a time sufficient to allow hybridization of said primer to the mitochondrial DNA; and

comparing an amount of hybridization of the oligonucleotide primer to the mitochondrial DNA of the first sample to an amount of hybridization of the primer to the mitochondrial DNA of the second sample, and therefrom determining the presence or absence of at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease.

22. The method of claim 21 wherein the mitochondrial single nucleotide polymorphism is located at a nucleotide that corresponds to a nucleotide position of SEQ ID NO:1 that is selected from the group consisting of position 72, 114, 146, 185, 189, 199, 204, 207, 228, 236, 239, 456, 462, 482. 489, 497, 500, 516, 522, 523, 547, 593, 669, 960, 1007, 1243, 1393, 1719, 1809, 2352, 2483, 2702, 2851, 3197, 3333, 3336, 3348, 3394, 3398, 3423, 3505, 3559, 3915, 3992, 4024, 4095, 4216, 4336, 4529, 4727, 4793, 4917, 4991, 5004, 5046, 5228, 5315, 5418, 5426, 5460, 5461, 5516, 5554, 5634, 5656, 5773, 6182, 6221, 6341, 6367, 6371, 6489, 7184, 7325, 7621, 7768, 7787, 7789, 7864, 7895, 7963, 8149, 8251, 8269, 8276-8284, 8470, 8485, 8508, 8602, 8697, 8752, 8901, 8994, 9123, 9254, 9362, 9380, 9477, 9554, 9708, 9804, 9861, 10034, 10044, 10238, 10463, 10589, 10978, 11065, 11251, 11253, 11272, 11470, 11527, 11611, 11674, 11812, 11914, 11947, 12414, 12501, 12609, 12705, 12954, 13111, 13194, 13212, 13368, 13617, 13780, 13966, 14020, 14148, 14178, 14179, 14182, 14212, 14233, 14470, 14582, 14905, 15028, 15043, 15191, 15299, 15380, 15553, 15607, 15758, 15790, 15808, 15833, 15884, 15924, 15928, 16069, 16086, 16093, 16126, 16129, 16145, 16147, 16172, 16174, 16182, 16183, 16189, 16192, 16193, 16223, 16224, 16234, 16235, 16239, 16248, 16256, 16261, 16270, 16278, 16290, 16292, 16293, 16294, 16298, 16300, 16304, 16309, 16311, 16320, 16355, 16362, 16391, 16482, 16524, 709, 930, 960, 980, 1189, 1243, 1700, 1719, 1809, 1811, 1888, 2098, 2158, 2259, 2352, 3010, 3197, 669, 789, 793, 870, 980, 1007, 1243, 1393, 1709, 1719, 2156, 2294, 2483, 2581, 2851, 6366 and 12954.

23. The method of claim 1 wherein the step of determining comprises contacting each of said first and second biological samples with a nucleic acid array comprising a plurality of isolated nucleic acid molecules immobilized on a solid support,

wherein said isolated nucleic acid molecules comprise all or a portion of the nucleic acid sequence set forth in SEQ ID NO:1 in which at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease is present, under conditions and for a time sufficient to allow hybridization of mitochondrial DNA to said isolated nucleic acid molecules; and

comparing an amount of hybridization of the mitochondrial DNA of the first sample to the nucleic acid array to an amount of hybridization of the mitochondrial DNA of the second sample to the nucleic acid array, and therefrom determining the presence or absence of at least one mitochondrial single nucleotide polymorphism that is associated with Alzheimer's disease.

24. The method of claim 23 wherein the mitochondrial single nucleotide polymorphism is located at a nucleotide that corresponds to a nucleotide position of SEQ ID NO:1 that is selected from the group consisting of position 72, 114, 146, 185, 189, 199, 204, 207, 228, 236, 239, 456, 462, 482. 489, 497, 500, 516, 522, 523, 547, 593, 669, 960, 1007, 1243, 1393, 1719, 1809, 2352, 2483, 2702, 2851, 3197, 3333, 3336, 3348, 3394, 3398, 3423, 3505, 3559, 3915, 3992, 4024, 4095, 4216, 4336, 4529, 4727, 4793, 4917, 4991, 5004, 5046, 5228, 5315, 5418, 5426, 5460, 5461, 5516, 5554, 5634, 5656, 5773, 6182, 6221, 6341, 6367, 6371, 6489, 7184, 7325, 7621, 7768, 7787, 7789, 7864, 7895, 7963, 8149, 8251, 8269, 8276-8284, 8470, 8485, 8508, 8602, 8697, 8752, 8901, 8994, 9123, 9254, 9362, 9380, 9477, 9554, 9708, 9804, 9861, 10034, 10044, 10238, 10463, 10589, 10978, 11065, 11251, 11253, 11272, 11470, 11527, 11611, 11674, 11812, 11914, 11947, 12414, 12501, 12609, 12705, 12954, 13111, 13194, 13212, 13368, 13617, 13780, 13966, 14020, 14148, 14178, 14179, 14182, 14212, 14233, 14470, 14582, 14905, 15028, 15043, 15191, 15299, 15380, 15553, 15607, 15758, 15790, 15808, 15833, 15884, 15924, 15928, 16069, 16086, 16093, 16126, 16129, 16145, 16147, 16172, 16174, 16182, 16183, 16189, 16192, 16193, 16223, 16224, 16234, 16235, 16239, 16248, 16256, 16261, 16270, 16278, 16290, 16292, 16293, 16294, 16298, 16300, 16304, 16309, 16311, 16320, 16355, 16362, 16391, 16482, 16524, 709, 930, 960, 980, 1189, 1243, 1700, 1719, 1809, 1811, 1888, 2098, 2158, 2259, 2352, 3010, 3197, 669, 789, 793, 870, 980, 1007, 1243, 1393, 1709, 1719, 2156, 2294, 2483, 2581, 2851, 6366 and 12954.

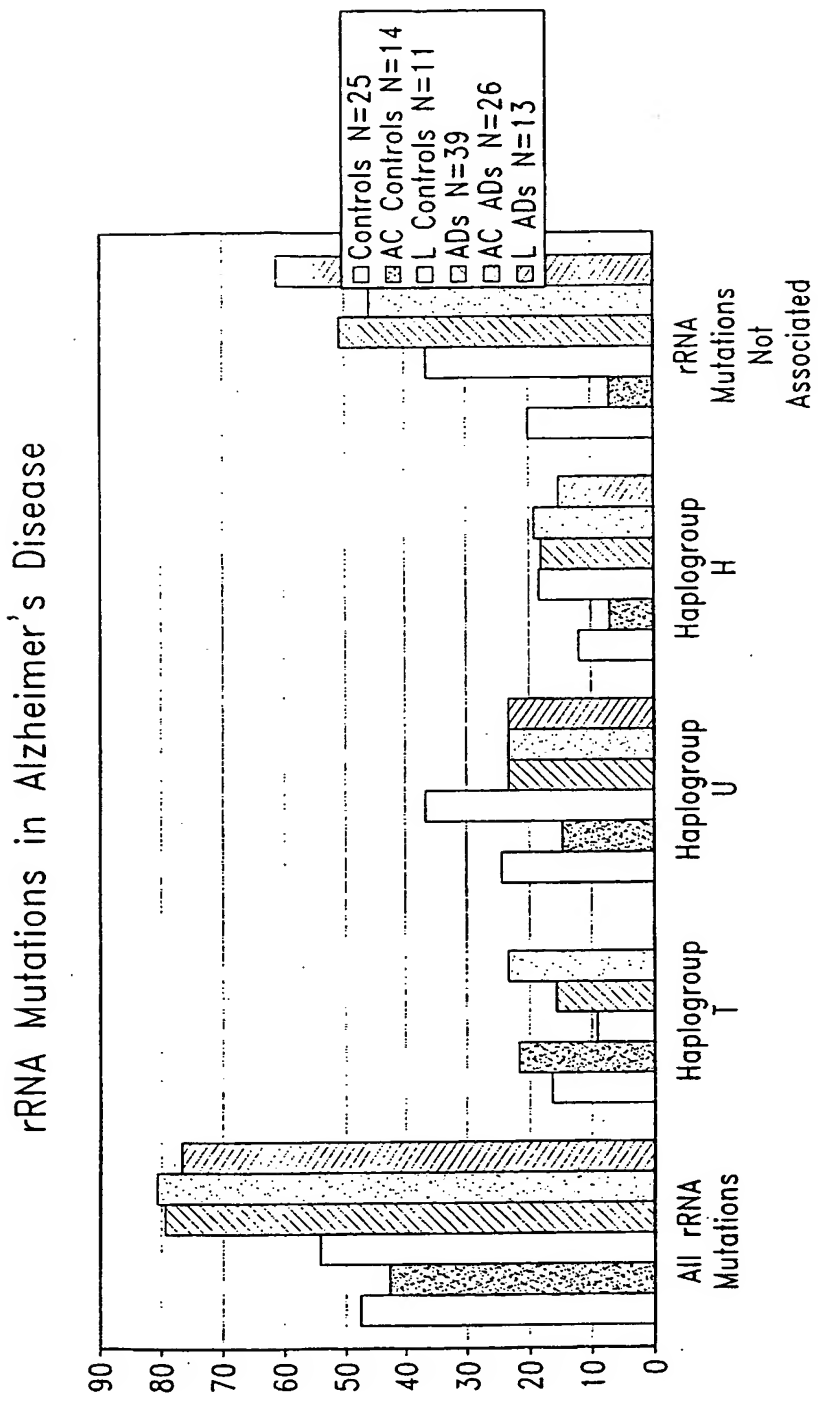
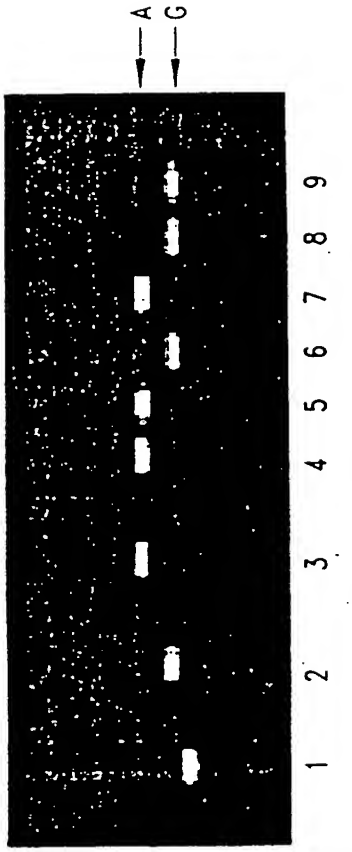

rRNA Mutations in Alzheimer's Disease
Controls N=25
AC Controls N=14
L Controls N=11
ADs N=39
AC ADs N=26
L ADs N=13
90
80
70
60
50
40
30
20
10
0
All rRNA Mutations
Haplogroup T
Haplogroup U
Haplogroup H
rRNA Mutations Not Associated

Fig. 1



(1) no template
(2) 6366 G DNA control
(3) 6366 A control
(4) DNA from AD-1 blood cells
(5) DNA from AD-1 cybrid cells
(6) DNA from SH-SY5Y cells
(7) cDNA from AD-1 cybrid cells
(8) cDNA from SH-SY5Y cells
(9) cDNA from pooled control cybrid cells

Fig. 2

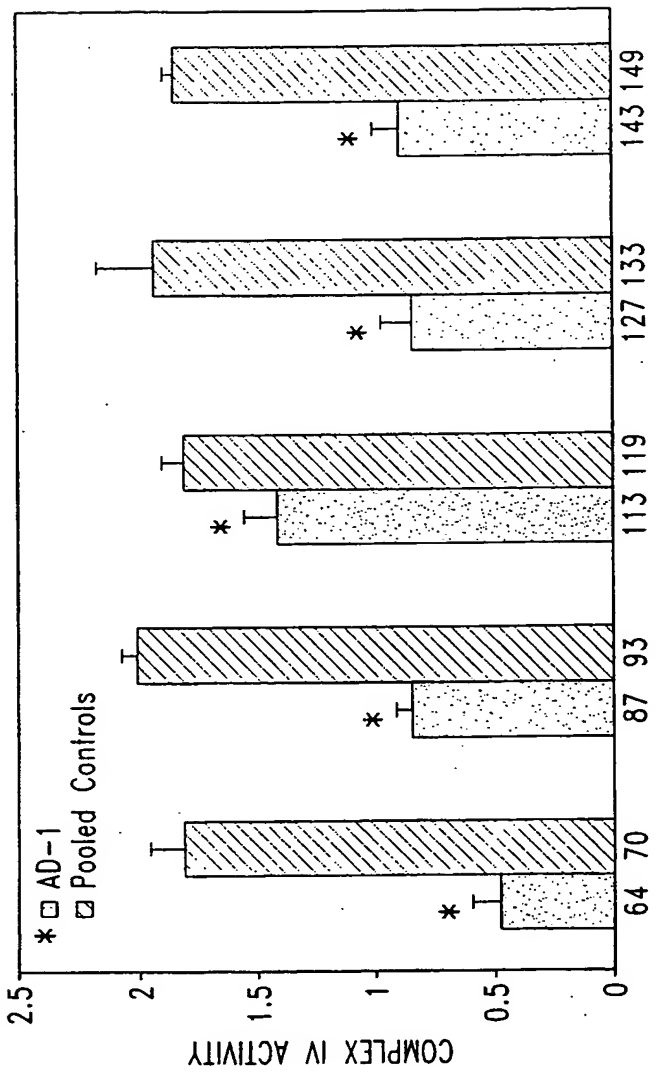

COMPLEX IV ACTIVITY
2.5
2
1.5
1
0.5
0
* AD-1
Pooled Controls
64 70
87 93
113 119
127 133
143 149
DAYS FROM FUSION

Fig. 3

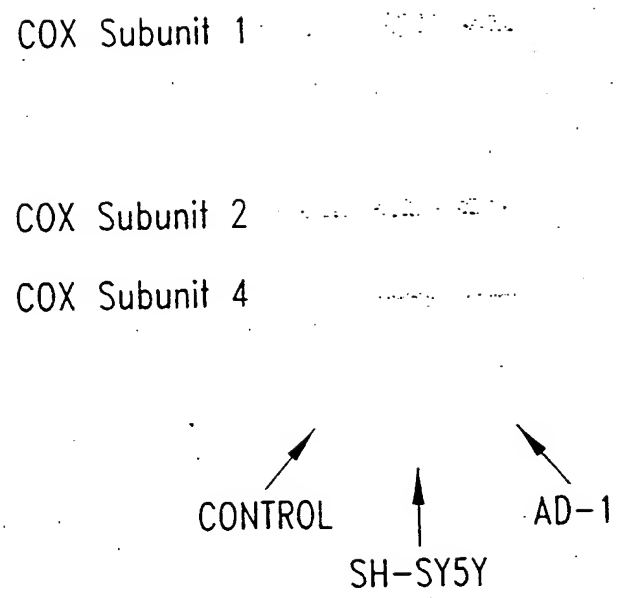


Fig. 4

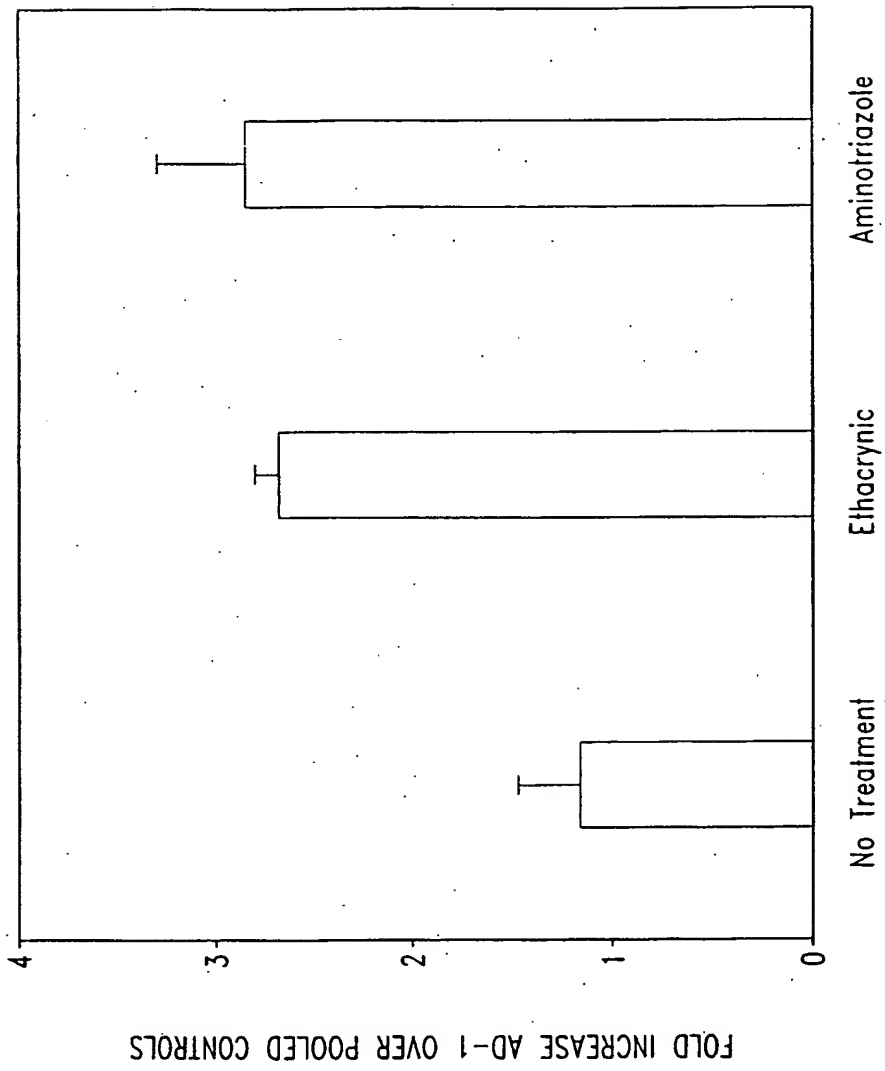

FOLD INCREASE AD-1 OVER POOLED CONTROLS
0
1
2
3
4
No Treatment
Ethacrynic Acid
Aminotriazole

Fig. 5

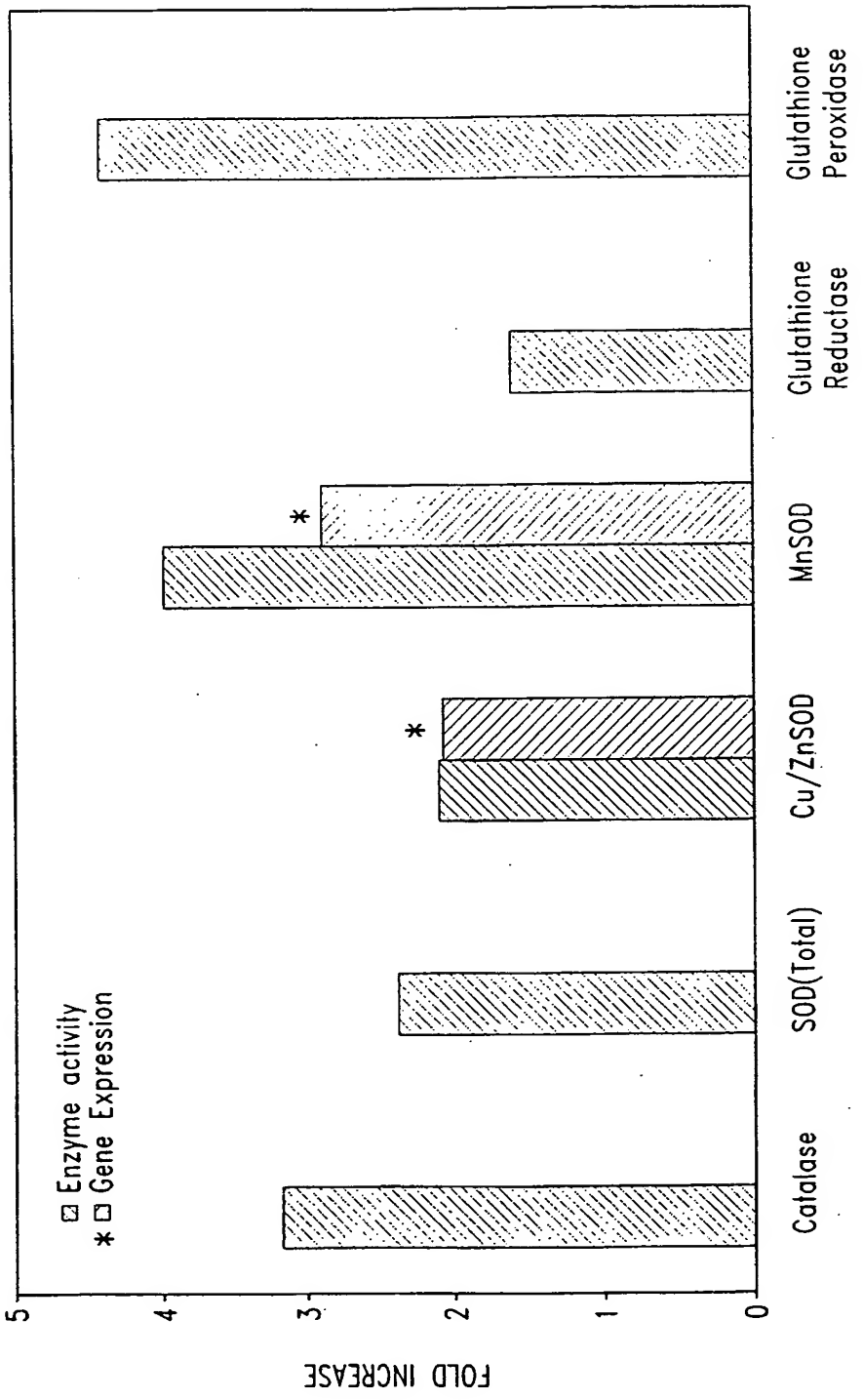

FOLD INCREASE
0
1
2
3
4
5
Enzyme activity
Gene Expression
*
*
Catalase
SOD(Total)
Cu/ZnSOD
MnSOD
Glutathione Reductase
Glutathione Peroxidase

Fig. 6

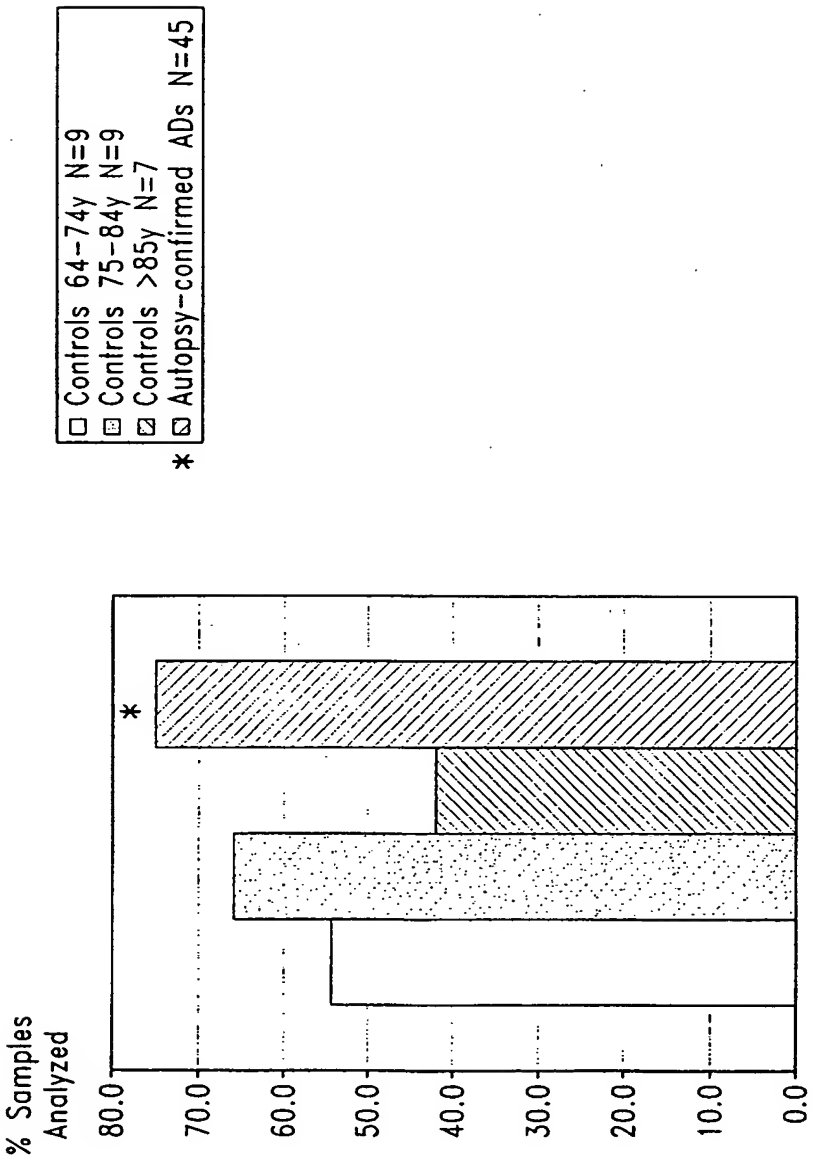

SNPs in the Mitochondrial rRNA Genes
Controls 64-74y N=9
Controls 75-84y N=9
Controls >85y N=7
Autopsy-confirmed ADs N=45
*
% Samples Analyzed
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0

Fig. 7

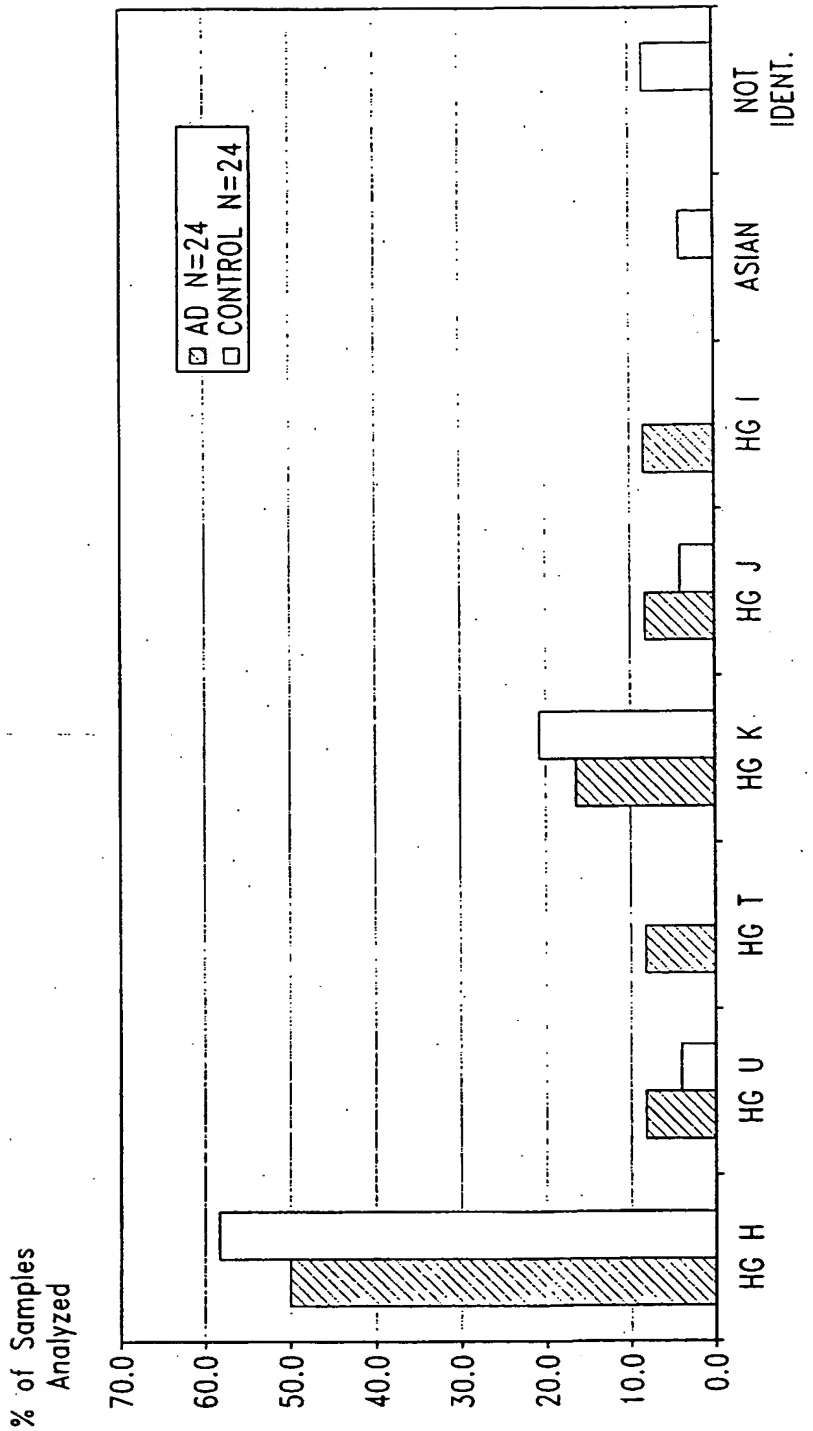


Fig. 8

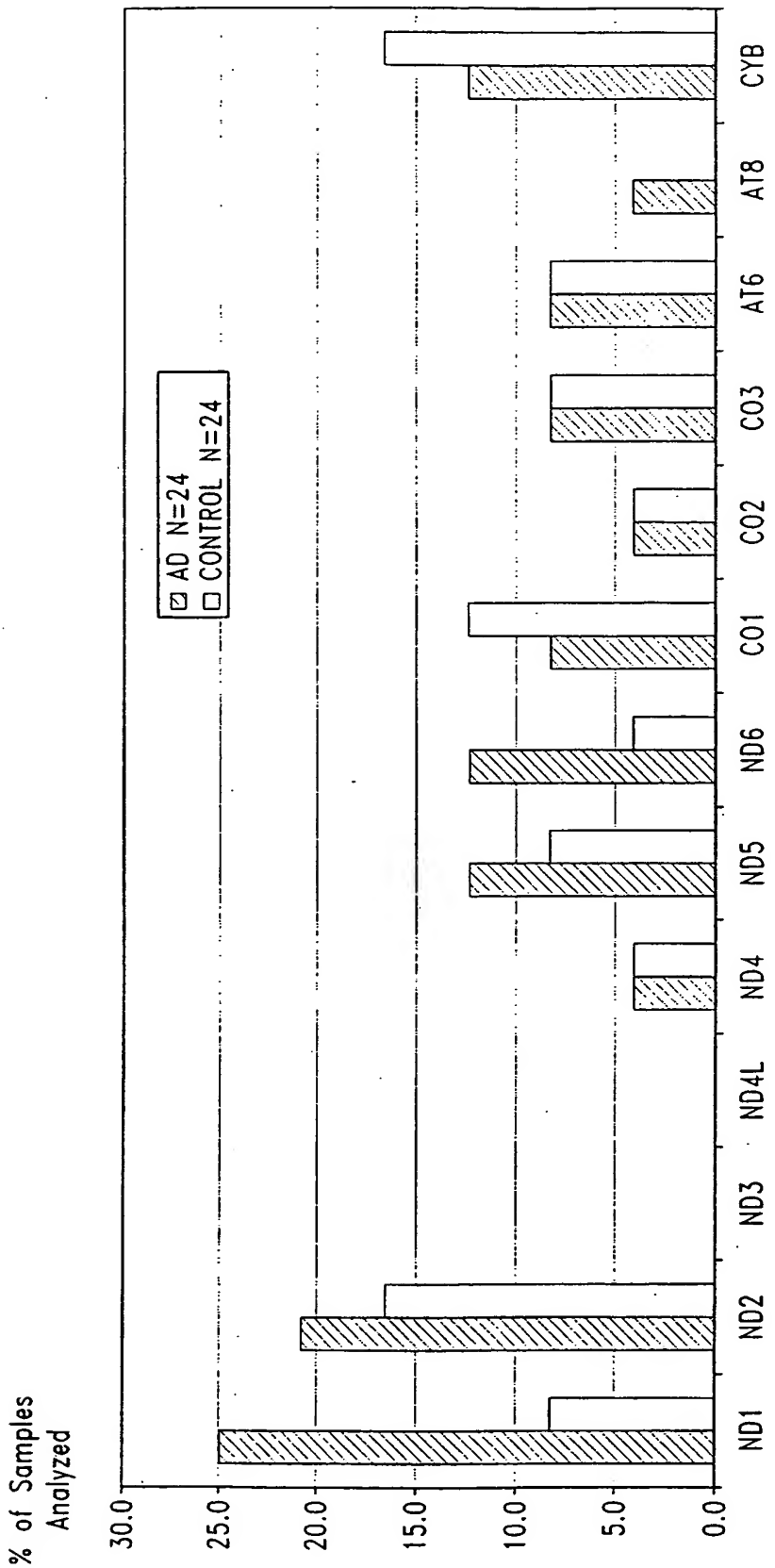

% of Samples Analyzed
30.0
25.0
20.0
15.0
10.0
5.0
0.0
AD N=24
CONTROL N=24
ND1
ND2
ND3
ND4L
ND4
ND5
ND6
CO1
CO2
CO3
AT6
AT8
CYB

Fig. 9

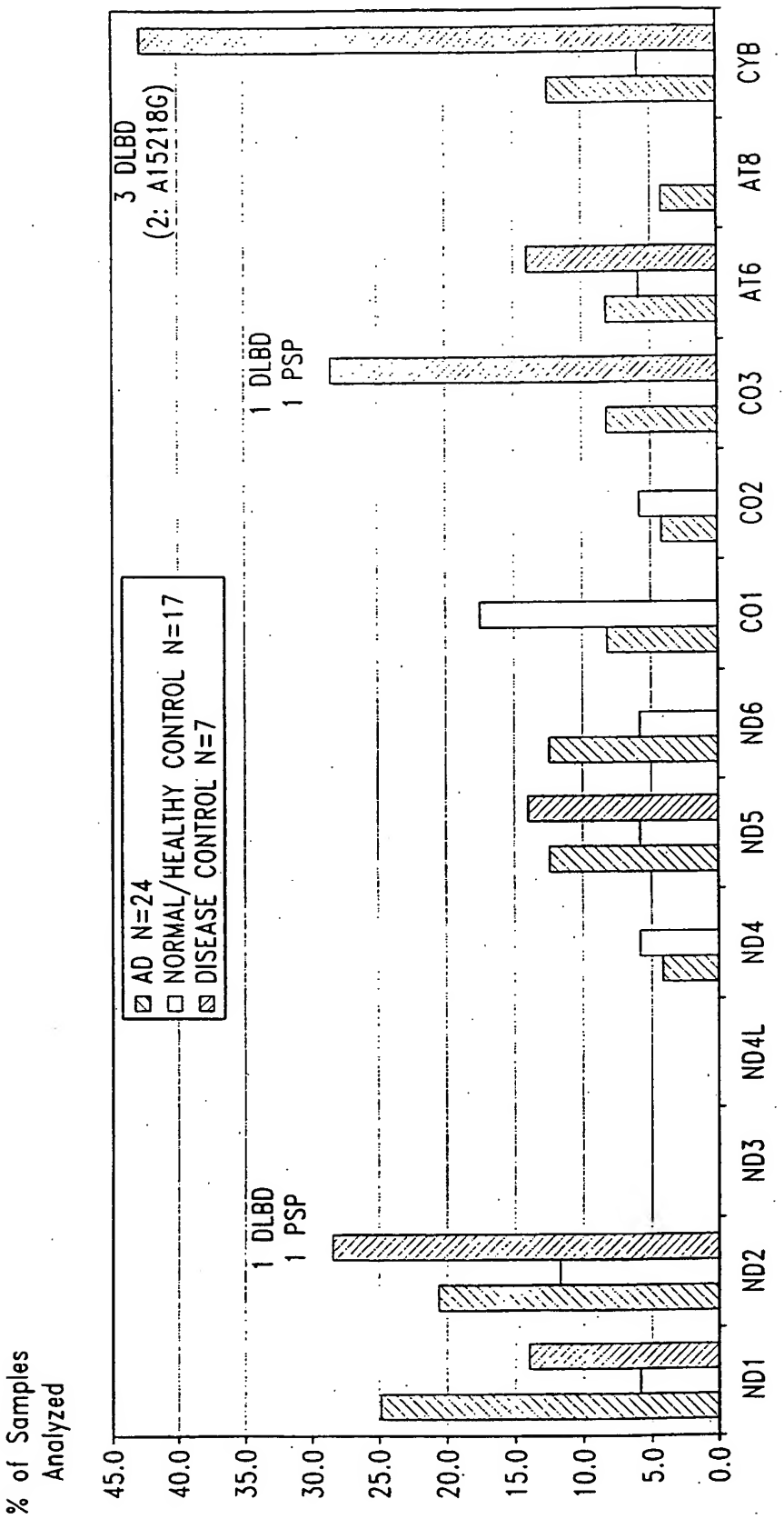

% of Samples Analyzed
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
AD N=24
NORMAL/HEALTHY CONTROL N=17
DISEASE CONTROL N=7
1 DLBD
1 PSP
1 DLBD
1 PSP
3 DLBD
(2: A15218G)
ND1
ND2
ND3
ND4L
ND4
ND5
ND6
CO1
CO2
CO3
AT6
AT8
CYB

Fig. 10

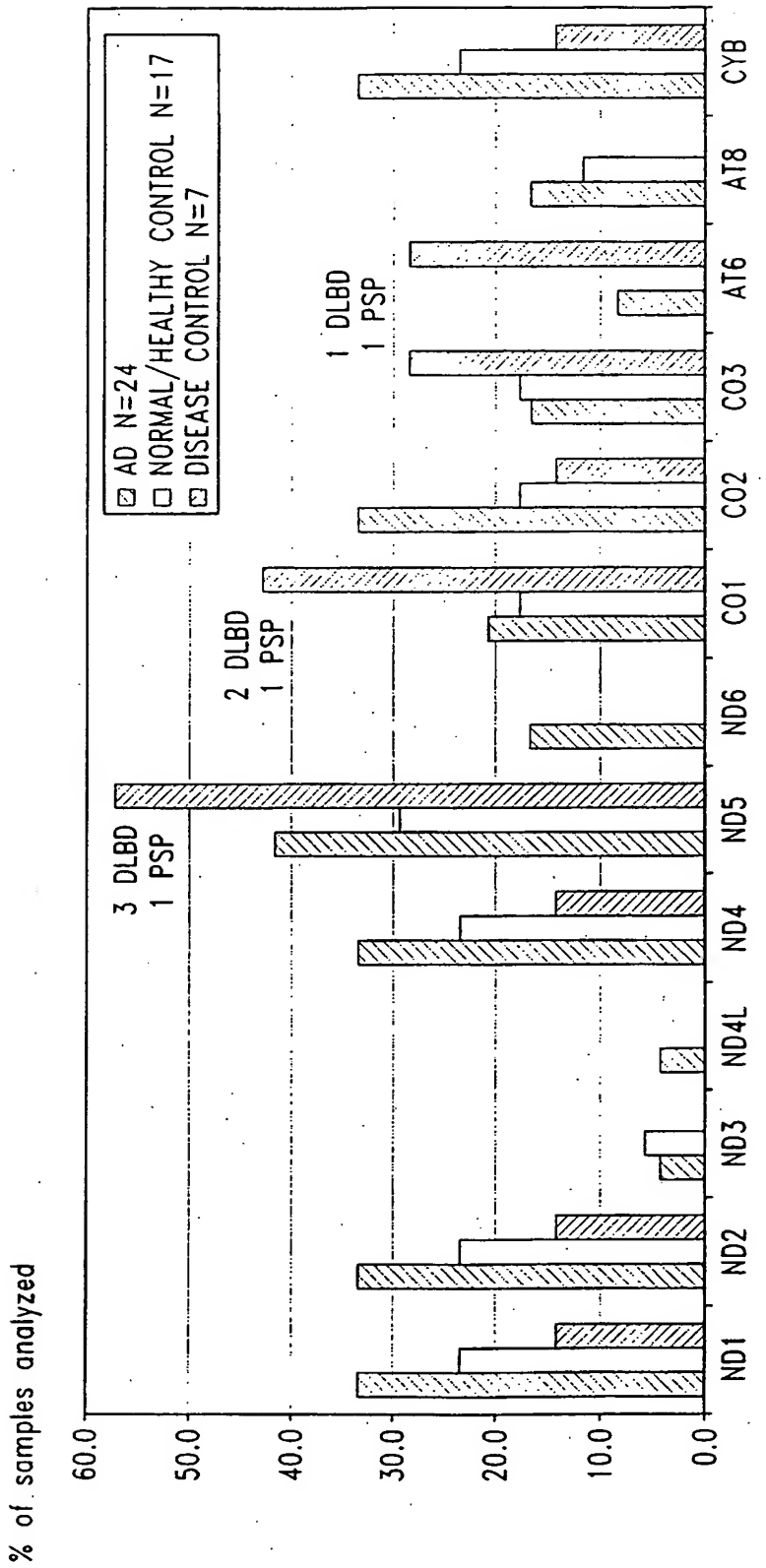

AD N=24
NORMAL/HEALTHY CONTROL N=17
DISEASE CONTROL N=7
3 DLBD
1 PSP
2 DLBD
1 PSP
1 DLBD
1 PSP
% of samples analyzed
60.0
50.0
40.0
30.0
20.0
10.0
0.0
ND1
ND2
ND3
ND4L
ND4
ND5
ND6
CO1
CO2
CO3
AT6
AT8
CYB

Fig. 11

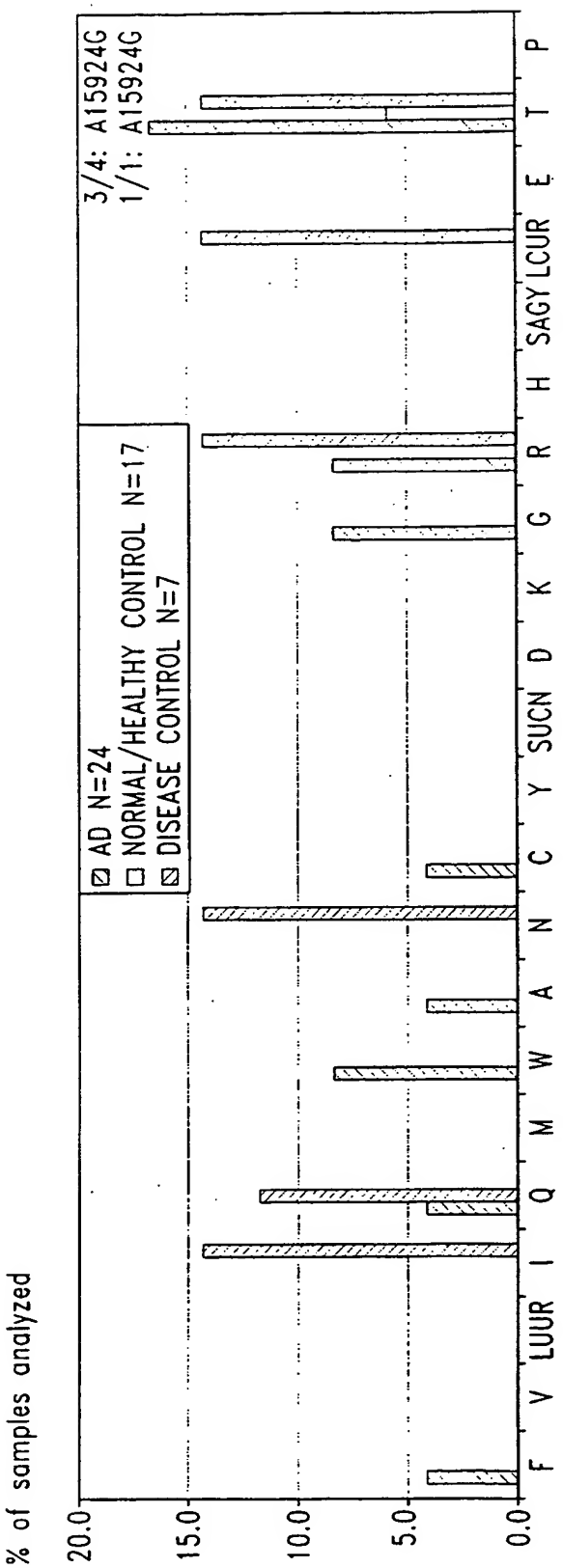

% of samples analyzed
20.0
15.0
10.0
5.0
0.0
AD N=24
NORMAL/HEALTHY CONTROL N=17
DISEASE CONTROL N=7
3/4: A15924G
1/1: A15924G
F V LUUR I Q M W A N C Y SUCN D K G R H SAGY LCUR E T P

Fig. 12

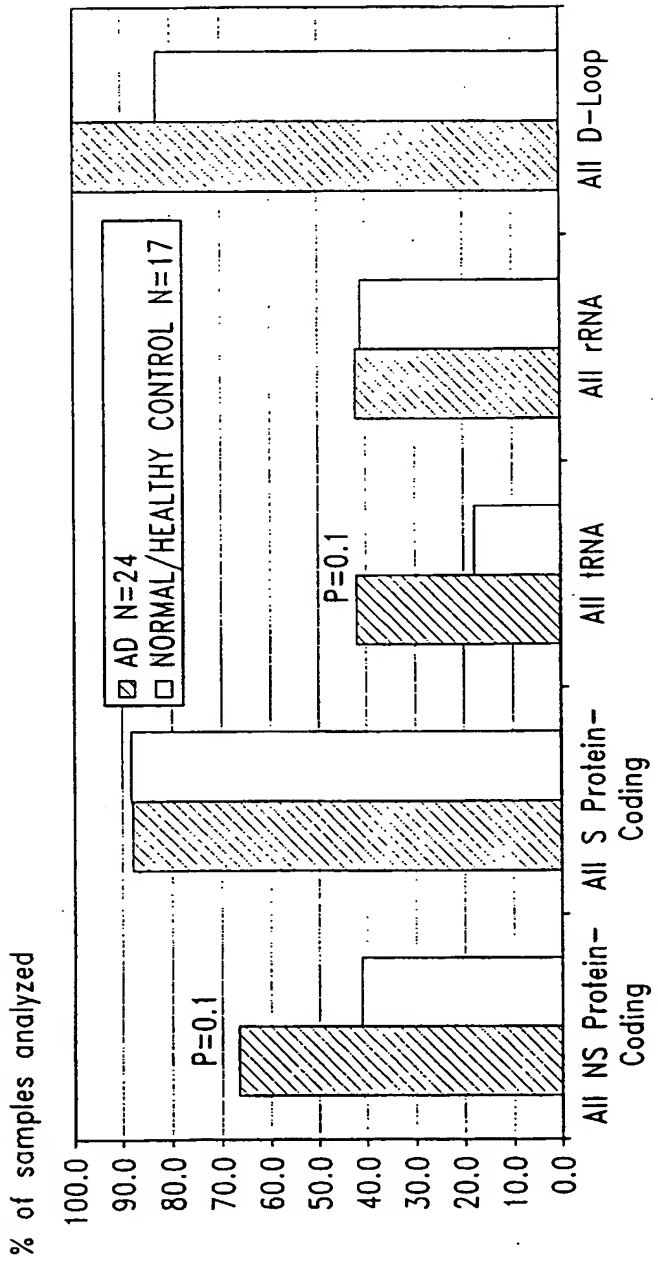

% of samples analyzed
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
AD N=24
NORMAL/HEALTHY CONTROL N=17
P=0.1
P=0.1
All NS Protein-Coding
All S Protein-Coding
All tRNA
All rRNA
All D-Loop

Fig. 13

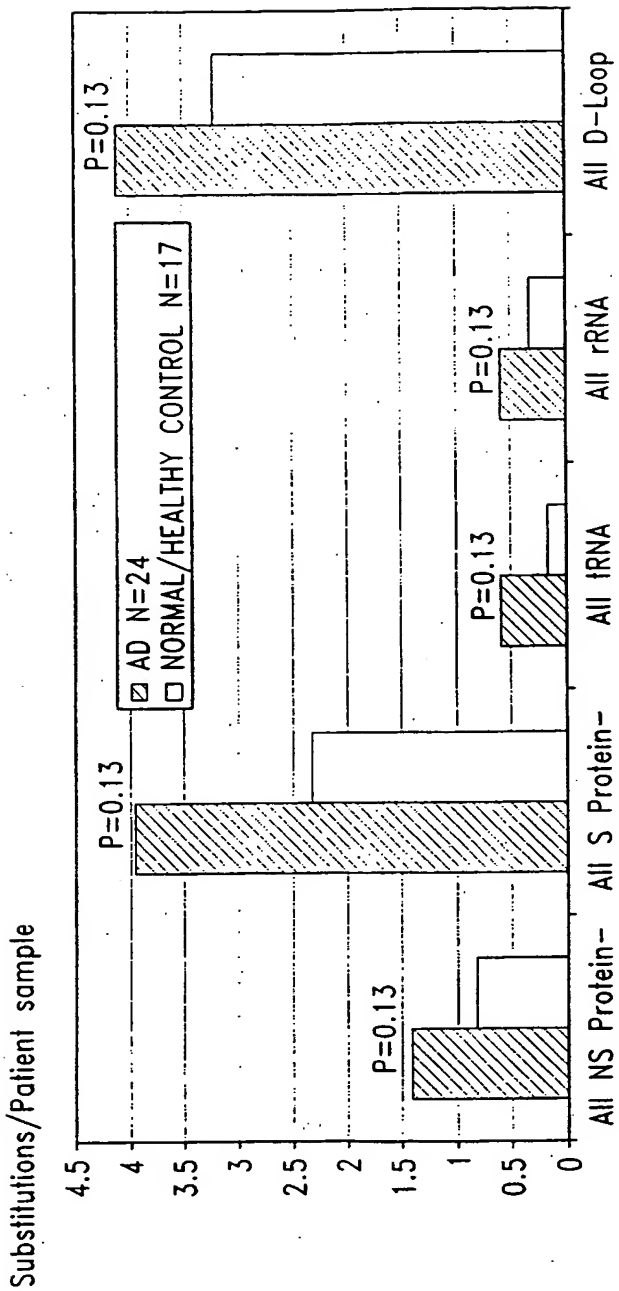

Substitutions/Patient sample
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
AD N=24
NORMAL/HEALTHY CONTROL N=17
P=0.13
P=0.13
P=0.13
P=0.13
All NS Protein-Coding
All S Protein-Coding
All tRNA
All rRNA
All D-Loop

Fig. 14

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(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **HERRNSTADT, Corinna** [DE/US]; 3628 Ruette DeVille, San Diego, CA 92130 (US). **DAVIS, Robert, E.** [US/US]; 13272 Glencliff Way, San Diego, CA 92130 (US).

(74) Agents: **ROSENMAN, Stephen, J.** et al.; Seed Intellectual Property Law Group PLLC, Suite 6300, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/63441 A3

(54) Title: SINGLE NUCLEOTIDE POLYMORPHISMS IN MITOCHONDRIAL GENES THAT SEGREGATE WITH ALZHEIMER'S DISEASE

(57) Abstract: Compositions and methods based on determination of single nucleotide polymorphisms in mtDNA or homoplasmic mtDNA mutations are provided that are useful for detecting the presence of or risk for having Alzheimer's disease (AD), and for identifying agents suitable for treating AD.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/10906

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, SEQUENCE SEARCH, WPI Data, PAJ, MEDLINE, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 38335 A (MITOKOR) 3 September 1998 (1998-09-03) the whole document ---	1-8, 11-14, 17-19, 21,23
X	WO 94 09162 A (UNIV EMORY MED) 28 April 1994 (1994-04-28) the whole document ---	1-8, 11-14, 17-19, 21,23
X	US 5 565 323 A (PARKER W DAVIS ET AL) 15 October 1996 (1996-10-15) cited in the application the whole document --- -/--	1-8, 11-14, 17-19, 21,23

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
3 September 2001	03. 12. 2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Knehr, M

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International Application No
PCT/US 00/10906

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FAHY E ET AL: "Multiplex fluorescence-based primer extension method for quantitative mutation analysis of mitochondrial DNA and its diagnostic application for Alzheimer's disease" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 25, no. 15, 1997, pages 3102-3109, XP002133461 ISSN: 0305-1048 cited in the application the whole document ---	1-8, 11-14, 17-19, 21,23
X	HUTCHIN T AND CORTOPASSI G: "A mitochondrial DNA clone is associated with increased risk for Alzheimer's disease" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA, vol. 92, 1995, pages 6892-6895, XP002176586 the whole document ---	1-8, 11-14,21
X	SHOFFNER J M ET AL.: "Mitochondrial DNA variants observed in Alzheimer Disease and Parkinson Disease patients" GENOMICS, vol. 17, 1993, pages 171-184, XP001023698 the whole document ---	1-8, 11-14
X	LIN F-H ET AL.: "Detection of point mutations in codon 331 of mitochondrial NADH dehydrogenase subunit 2 in Alzheimer's brains" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 182, no. 1, 1992, pages 238-246, XP002176587 the whole document ---	1-8, 11-14
X	CHAGNON P ET AL.: "Brain mitochondrial DNA polymorphism and cytochrome oxidase activity in Alzheimer's disease" ALZHEIMER'S RESEARCH, vol. 2, 1996, pages 237-242, XP001023664 the whole document ---	1-8, 11-14
X	TANNO Y ET AL.: "mtDNA polymorphisms in Japanese sporadic Alzheimer's disease" NEUROBIOLOGY OF AGING, vol. 19, no. 1S, 1998, pages S47-S51, XP001023696 the whole document --- -/--	1-8, 11-14

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/10906

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 812 922 A (AFFYMETRIX INC) 17 December 1997 (1997-12-17) the whole document ---	11-14,19
A	WO 98 23632 A (MITOKOR) 4 June 1998 (1998-06-04) the whole document ---	
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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/10906

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1,5-16,19-24 (partially); 2-4,17,18 (complete)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: Invention 1: Claims 1,5-16,19-24 (partially); 2-4, 17,18 (complete)

INVENTION 1:
Methods for determining the risk for or presence of Alzheimer's disease by determining the presence or absence of a mitochondrial single nucleotide polymorphism corresponding to position 72 of SEQ ID NO:1, methods of determining whether an agent causes, contributes, or exacerbates Alzheimer's disease via such an polymorphism, as well as an array of nucleic acids comprising such an polymorphism, suitable within such methods.

2. Claims: Inventions 2-213: Claims 1,5-16,19-24 (partially)

INVENTION 2 TO INVENTION 213:
Methods for determining the risk for or presence of Alzheimer's disease by determining the presence or absence of a mitochondrial single nucleotide polymorphism corresponding to positions 114, 146, 185, ..., 16391, 16482, and 16524, as well as positions 709, 930, 980, 1189, 1700, 1811, 1888, 2098, 2158, 2259, 3010, 789, 793, 870, 1709, 2156, 2294, 2581, and 6366, of SEQ ID NO:1, methods of determining whether an agent causes, contributes, or exacerbates Alzheimer's disease via such an polymorphism, as well as an array of nucleic acids comprising such an polymorphism, suitable within such methods.

Invention 2 refers to polymorphism 114,

ibidem
...

Invention 3 refers to polymorphism 146,
Invention 4 refers to polymorphism 185,
...
Invention 193 refers to polymorphism 16482,
Invention 194 refers to polymorphism 16524,

Invention 195 refers to polymorphism 709,
Invention 196 refers to polymorphism 930,
...
Invention 212 refers to polymorphism 2581,
Invention 213 refers to polymorphism 6366.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/10906

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